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Assessment of Organophosphorous Pesticide Residues in Dried Cocoa Beans from Selected Cocoa Growing Zones of Nigeria.

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ABSTRACT
Determination of pesticide residues in food is very essential in human health and it is becoming a major priority in the cocoa value chain. Hence, organophosphorous pesticide residues in dried cocoa beans from Ondo and Osun States, Nigeria were determined using Gas Chromatography – Mass Spectrometry (GC-MS) and the detected levels of these residues were compared with the European Union Maximum Residue Levels (EU MRLs). Samples were milled separately and 10 g milled samples were weighed into 40 ml Dichloromethane (DCM) in a beaker and later sonicated. The extracts were subjected to gel permeation chromatography to separate lipids from the extracts, which were later subjected to fractionation. Among the organophosphous pesticide residues detected in the samples from the states were dichlorvos, diazion, phosphamidon, pirimiphos-methyl, chlorpyrifos, isofenphos and carbofenothion with their being levels higher than EU MRLs, suggesting that the produce were not safe for human consumption and the residues could pose some health risk to the consumers.

Introduction

Nigeria is one of the principal producers of cocoa and has risen as a major exporter of the product over the last century. She is ranked among the five largest producers in the world behind Côte d'Ivoire Ghana and Indonesia. Cocoa production in Nigeria is essentially on a small-scale level and is mainly produced in fourteen different states, with huge production from Ekiti, Ondo, Osun, Oyo and Ogun (ICCO, 2012). In 2013, Nigeria alone produced 300,000 tonnes of the world's cocoa sowing an increase of seven percent compared to the previous year's (USDA, 2014). Therefore, cocoa produce is taking the lead as the non-oil export product in Nigeria.

Mirid and capsid are general terms used to refer to several species of insect pests found in cocoa growing regions. Different species are found in different regions, but the effects are similar. Globally, mirids account for 200,000 MT of losses (World Cocoa Foundation, 2014) and are the most serious pests of cocoa in West Africa (Opeke, 1992). A species of mirid is the brown cocoa mirid (*Sahlbergella singularis*) which is very prominent in West Africa (Anikwe, 2008), while the second specie is the black mirid (*Distantiella theobroma*) (Opeke, 1992). The brown mirid is very common and attack cocoa in all parts of West Africa by feeding on the pods, chupons and branches with the winged adult having the potentials of feeding on hard woods (Opeke, 1992). Although black pod is the greatest threat in West Africa, brown cocoa mirids can also cause significant losses (World Cocoa Foundation, 2014). Mirids in both the nymph and adult phases feed on the pods, trunks and twigs (Anikwe, 2008). They leave behind lesions which can be infected by parasitic fungi that cause dieback of twigs and cankers on the trunk. The first year a farm is infested by mirids, a 30% loss in production could be recorded while in subsequent years, increase in the mirid population usually increase the damage to the trees as well as the losses rise to 75% (Anikwe, 2008).

Attempt to reduce cocoa insect pests requires the use of insecticides of various kinds both in the field and storage. Pesticides are a group of chemicals made for the purpose of killing or otherwise deterring "pest" species. The word *pesticide* may refer to insecticides, herbicides, fungicides, or other pest control formulations. Pesticides are inherently toxic and often associated with adverse health effects in non-target organisms (USDA, 2015). While some pesticides have long residual activity and therefore persist in the environment, others have short residual activity, disappear from the environment or produce low residue concentration. Pesticide residues on crops are monitored with reference to Maximum Residue Limits (MRLs) and are based on analysis of quantity of a given Active Ingredient (AI) remaining on food product samples.

Since the removal of OCPs from use, OPs have become the most widely used insecticides. Among all organophosphorous (OP) pesticides used in Nigeria, Dichlorvos, which is sold as DDforce and Chlorpyrifos, which is sold as Rocket are more prominent (Ogunfowokan et al., 2012). Organophosphorous pesticides degrade rapidly by hydrolysis on exposure to sunlight, air, and soil, although small amounts can be detected in food and drinking water. Many metabolites of organophosphorous are more toxic than the primary chemicals themselves, making them acutely lethal to sensitive species like amphibians (USGS, 2007). Major metabolites of chlorpyrifos and malathion were 100 times more toxic than their parent compounds to frogs in California's Central Valley, where nearly 25% of the nation's organophosphorous used is concentrated (USEPA, 2007). Because organophosphorous share a common toxicity mechanism, exposure to several OP insecticides and their breakdown products could intensify their toxic effects.

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OPs share a common mechanism of cholinesterase inhibition and poison insects and mammals by phosphorylation of AChE enzyme nerve endings (USEPA, 2007).

Some of the efforts in monitoring pesticide residues in food in the country have revealed the presence of dichlorodiphenyltrichloroethane (DDT) and its metabolites in cocoa beans (Aikpokpodion et al., 2012a); α-HCH, Lindane, Dieldrin, Endrin and β-HCH in cocoa dried beans (Olaniran et al., 2016); blood serum of cocoa famers and drinking water of cocoa farming communities (Sosan et al., 2008) and cowpea grains and yam chips (Olufade et al., 2014). This present study is however aimed at investigating the presence and the levels of organophosphorous insecticide residues in dried cocoa beans in selected stores from Osun and Ondo states, Nigeria.

Materials and methods

Sample collection and preparation

Dried cocoa beans samples were collected from six different cocoa stores in Ile-Ife and Ilesa (Osun state) and Ondo and Idanre (Ondo state) in January, 2014. The two states were chosen because of production capacities. Ondo state is the top producer of cocoa in Nigeria with the capacity to produce about 90,000 MT of cocoa bean between 2006-2010 production years, and Osun state with the capacity of producing 75,000MT. Two kilograms each of dried cocoa beans were randomly taken from bags of cocoa beans in the stores. Samples were dried to constant weight and thoroughly milled using a kitchen blender.

Extraction and clean-up procedures

10 g of each sample was accurately weighed using sensitive scale into 60 mL volume beaker. Following the extraction procedure used in (Olaniran et al., 2016). 40 mL dichloromethane (DCM) was added to already weighed samples and subjected to sonication for 30 min in a (360 W) Selecta Ultrasonic Bath. Pre-sonicated Watman 1 filter paper was used to separate the filtrate.

Gel permeation chromatography was applied to the extracts using bio-beads S-X3 pack glass column (380 x 22 i.d) with 300 mL hexane: dichloromethane in 1:1 volume. The lipid content was collected and discarded. The leftover of the filtrate was collected, concentrated and divided into the pesticides and saturates. Extracts were later subjected to elution using glass column packed with silica-alumina at 2:1 into two fractions of hydrocarbon and the pesticides, in 7:3 volumes of hexane/dichloromethane. The resulting elutes were concentrated at 30^oC to 1 mL, using rotary evaporator and transferred into 2 mL volume pre-sonicated chromatographic vials and evaporated to 0.5 mL under a stream of analytical grade nitrogen.

Gas chromatographic analysis

The detection of organophosphorous insecticide residues was carried out on Gas Chromatograph equipment coupled to Mass Spectrometry (GC-MS) using Shimadzu Model Q 2010 GC-MS (Shimadzu, Japan) fitted with HP-5MS column of fused silica (30 x 0.25 x 0.25 mm i.d. film thickness) and the analytical grade helium (99.99 %) was used as carrier gas. The injection mode was split and the split time was 1 min after injection using auto-sampler and the injection temperature was 250^oC. The temperature was programmed from 80^oC (held for 1 min) to 180^oC at 10^oC/min (held for 2 min) and to 300^oC at 10^oC/min (held for 2min). The residues of were identified by comparing the retention time of sample peaks with that of the pesticide grade standards.

Results

Levels of organophosphate insecticide residues in dried cocoa bean samples from Osun state compared with the European Union Maximum Residue Levels (EU MRLs) were presented in Table 1. Samples from Ile-Ife, Osun state contained the residues of diazinon (0.098 mg/kg), phosphamidon (0.146 mg/kg) and pirimiphos-methyl (0.495 mg/kg) at levels being higher than their respective EU MRLs (0.05 mg/kg, 0.02 mg/kg and 0.05 mg/kg). Levels of pirimiphos-methyl and phosphamidon from the town were extremely higher than their respective EU MRLs. The organophosphorous insecticide residues detected in samples from Ilesa include diazion (0.089 mg/kg), phosphamidon (0.110 mg/kg), chlorpyrifos (0.245 mg/kg), isofenphos (0.122 mg/kg) and carbofenothion (0.098 mg/kg) with their concentration levels higher than their respective EU MRLs (0.05 mg/kg, 0.02 mg/kg, 0.10 mg/kg, 0.01 mg/kg and 0.01 mg/kg) (Table 1). Figures 1 and 2 present the chromatographs of the detected peaks of organophosphate insecticide residues in Osun state.

Concentration levels of organophosphate pesticide residues in dried cocoa bean samples from Ondo state compared with the EU MRL were presented in Table 2. Residues of dichlorvos (0.088 mg/kg), diazinon (0.094 mg/kg), chlorpyrifos (0.062 mg/kg), parathion (0.168 mg/kg), isofenphos (0.108 mg/kg), bromophos-ethyl (0.224 mg/kg) and carbofenothion (0.227 mg/kg) were found in samples from Ondo town of the State. Dichlorvos, diazinon, parathion, isofenphos, bromophos-ethyl and carbofenothion were found with levels higher than their respective EU MRLs (0.02 mg/kg, 0.05 mg/kg, 0.10 mg/kg, 0.01 mg/kg, 0.10 mg/kg and 0.01 mg/kg), with only chlorpyrifos being lower than the EU MRL (0.10 mg/kg). Samples from Idanre in Ondo state contained residues of dichlorvos (0.086 mg/kg), mevinphos (0.106 mg/kg), diazinon (0.109 mg/kg), dichlofenthion (0.144 mg/kg), chlorpyrifos (0.162 mg/kg),

Table 1. Concentration of organophosphate pesticide residues in dried cocoa bean samples from Osun State.

Pesticides	Retention Time Ile-Ife	Retention Time Ilesa	Conc. (mg/kg) Ile-Ife	Conc. (mg/kg) Ilesa	EU MRL (mg/kg)
Dichlorvos	ND	ND	ND	ND	0.02
Mavinphos	ND	ND	ND	ND	0.02
Dimethoate	ND	ND	ND	ND	0.05
Diazinon	14.188	14.275	0.098	0.089	0.05
Phosphamidon	15.272	15.286	0.146	0.110	0.02
Dichlofenthion	ND	ND	ND	ND	0.05
Pirimiphos-methyl	16.242	ND	0.495	ND	0.05
Chlorpyrifos	ND	16.799	ND	0.254	0.10
Parathion	ND	ND	ND	ND	0.10
Isofenphos	ND	17.758	ND	0.122	0.01
Bromophos-ethyl	ND	ND	ND	ND	0.10
Ethion	ND	ND	ND	ND	0.05
PF-38	ND	ND	ND	ND	0.01
Carbofenothion	ND	20.565	ND	0.098	0.01

ND= Not Detected

Table 2. Concentration of organophosphate pesticide residues in dried cocoa bean samples from Ondo State.

Pesticides	Retention Time Ondo	Retention Time Idanre	Conc. (mg/kg) Ondo	Conc. (mg/kg) Idanre	EU MRL (mg/kg)
Dichlorvos	6.633	6.620	0.088	0.086	0.02
Mavinphos	ND	9.204	ND	0.106	0.02
Dimethoate	ND	ND	ND	ND	0.05
Diazinon	14.186	14.352	0.094	0.109	0.05
Phosphamidon	ND	ND	ND	ND	0.02
Dichlofenthion	ND	15.468	ND	0.144	0.05
Pirimiphos-methyl	ND	ND	ND	ND	0.05
Chlorpyrifos	16.727	16.890	0.062	0.162	0.10
Parathion	17.087	17.030	0.168	0.181	0.10
Isofenphos	17.753	17.754	0.108	0.131	0.01
Bromophos-ethyl	18.291	18.291	0.224	0.295	0.10
Ethion	ND	ND	ND	ND	0.05
PF-38	ND	ND	ND	ND	0.01
Carbofenothion	20.564	20.563	0.227	0.172	0.01

ND= Not Detected

parathion (0.181 mg/kg), isofenphos (0.131 mg/kg), bromophos-ethyl (0.295 mg/kg) and carbofenothion (0.172 mg/kg) with levels being higher than their respective EU MRLs (0.02 mg/kg, 0.02 mg/kg, 0.05 mg/kg, 0.05 mg/kg, 0.10 mg/kg, 0.10 mg/kg, 0.01 mg/kg, 0.10 mg/kg and 0.01mg/kg respectively) as presented in Table 2. Figures 3 and 4 present the chromatographs of the detected peaks of organophosphate pesticide residues in Ondo and Idanre towns.

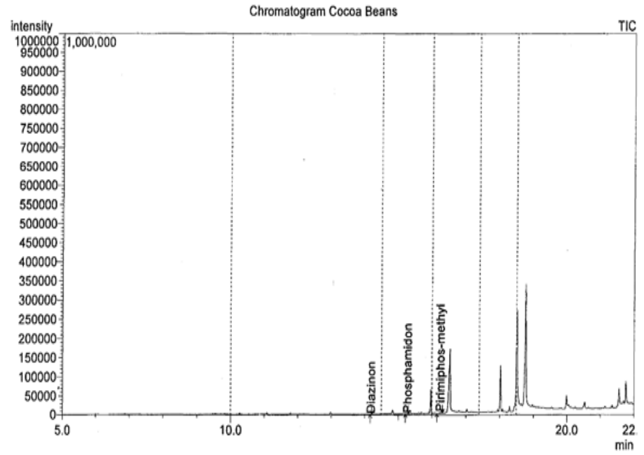

Figure 1.The chromatograph of organophosphate pesticides peaks in dried cocoa beans samples from Ile-Ife town.

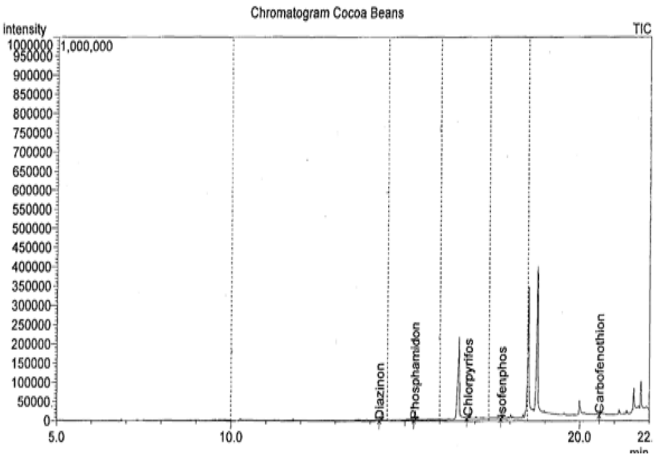

Figure 2.The chromatograph of organophosphate pesticides peaks in dried cocoa beans samples from Ilesa town .

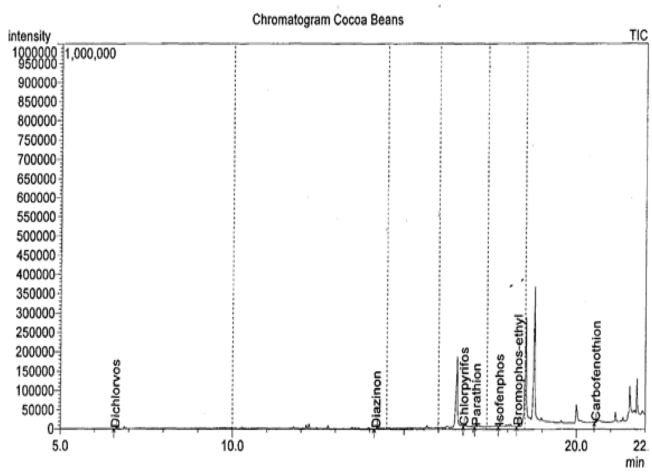

Figure 3.The chromatograph of organophosphate pesticides peaks in dried cocoa beans samples from Ondo town.

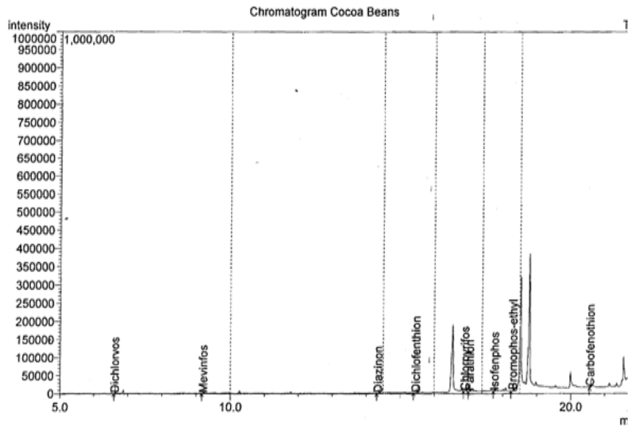

Figure 4.The chromatograph of organophosphate pesticides peaks in dried cocoa beans samples from Idanre town.

Discussion

The organophosphorous insecticide residues detected in samples from Osun state were diazinon, phosphamidon, pirimiphos-methyl, chlorpyrifos, isofenphos and carbofenothion. Among these residues found, diazinon, pirimiphos-methyl and chlorpyrifos are World Health Organization (WHO) category II pesticides, which are moderately hazardous with acute oral LD>50<500 (WHO, 2010). Isofenphos and carbofenothion are classified by (WHO, 2010), as obsolete pesticides, while phosphamidon belong to category Ia (extremely hazardous) pesticide with acute oral LD≤5 by the same classification. All the levels of residue found in samples from Osun state were higher than their respective EU-MRLs. By implication, the organophosphorous insecticide residues found in samples from Osun state may hinder the produce acceptability in the European Union markets.

Dichlorvos, mavinphos, diazinon, dichlofenthion, chlorpyrifos, parathion, isofenphos, bromophos-ethyl and carbofenothion were the residues of organophosphorous insecticide residues found in samples from Ondo state. Similar to the levels detected in sample from Osun state, diazinon, chlorpyrifos, isofenphos and carbofenothion were also found in samples from Ondo state at various levels being higher than the EU MRLs, but with chlorpyrifos from Ondo town being lower than the EU MRL. These suggest that similar pesticides are used by cocoa farmers in the two states. Ondo and Osun states are two neighbouring states in the south-western Nigeria that fall in the same agro-ecological zone and grow similar cash crops. This might be the reason for using similar pesticides in cocoa production in the two states. Levels of dichlorvos, mavinphos, dichlofenthion, parathion and bromophos-ethyl were also detected but only in samples from Ondo state, which were higher than their respective EU MRLs.

Diazinon, chlorpyrifos and dichlorvos are active ingredients of most organophosphorous insecticides used in controlling insect pests of cocoa in Nigeria. Chlorpyrifos is sold as Durban 48EC in Nigeria and it is an approved insecticide used in controlling cocoa mirid and termite in cocoa plantations (Mokwunye et al., 2010). Mirid and termite have been major constraint to cocoa production in the two states under this study and organophosphorous insecticides have been used in their control (Aikpokpodion et al., 2011b). Highest levels of concentration of these insecticides detected in the towns from the two states were higher than the EU MRL. This suggests that the insecticides are indiscriminately used in the states. The insecticides, which kills by altering normal neurotransmission within the nervous system of target organism (USEPA, 2007a) and their toxicity to non-target organisms is similar to that of the target organisms (USEPA, 2007b). The insecticides are also fat soluble which in turns may result in delayed toxicity especially if significant amounts are stored in fatty tissues of man or livestock (USEPA, 2007b). This explains the potential of theseinsecticides to be chronically toxic to man and livestock. However, diazinon is moderately persistent and mobile when released into the environment (United Nation Environmental Programme, 2001).

Levels of dichlorvos were registered in the two towns (Ondo and Idanre) of Ondo state but absent in Osun state with the highest found in samples from Ondo town being higher than the EU-MRL. Absence of dichlorvos level in samples from Osun state reveals that cocoa farmers in the state may not be applying the pesticide in cocoa production. Dichlorvos is a class Ib pesticide (Highly hazardous) with acute LD > 5 < 50 (WHO, 2010) and was listed among the suggested pesticides not to be used for cocoa production (Bateman, 2010). Dichlorvos, when compared with poisoning by other organophosphorous pesticides causes a more rapid onset of symptoms followed by a rapid recovery (Erdman, 2004). This suggests that dichlorvos could be rapidly metabolized and excretes from human being and livestock. The pesticide has been proposed for cancellation since 1995 by Environmental Protection Agency of the United State for many commercial and industrial uses (ATSDR, 1997). Human exposure to the insecticide could include home, place of work, food and warehouse fumigation which could result in cholinergic signs such as tremors and diarrhea and death in experimental animals (Elersek and Filipic, 2011). The chronic health hazard to humans from repeated exposures to dichlorvos was evaluated based on the inhibition of cholinesterase activity in the brain observed in both inhalation and oral studies, which could result in cancer risk (Elersek and Filipic, 2011).Organophosphate pesticides are generally enzyme acetylcholinesterase (AChE) inhibitors and the measurement of human exposure to such pesticides is determined by the increase or decrease in AChE activity (Lori et al., 1996). Farmers and consumers of cocoa beans produced from the study areas may face some health challenges earlier stated on long term exposure to dichlorvos.

Conclusion

This study has shown that residues of organophosphorous pesticides are present dried cocoa beans samples from the two states. Also, the levels of the insecticide residues detected were above the EU MRLs suggesting that the produce were unsafe for human consumption. Prompt monitoring of pesticide residues should however be embarked upon on the produce to ensure their reduction in the value chain. Efforts should also be made to regulate importation of pesticides into the country as well as to strictly enforce laws on their usage.

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