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Article

Level of Organochlorine and Pyrethroid Pesticide Residues in Vegetable Farmers' Blood of Southern Benin

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Abstract Pesticides are a class of chemical substance that can destroy pests and other unwanted organisms. When sprayed by the farmers on their crops against pests, pesticides can also have an adverse effects on human health. Determination of pesticide residues in human blood is one of the methods used to evaluate their exposure. Despite pesticides being used by farmers in Benin, there has been no data on the levels of organochlorine (OC) and pyrethroid (Pyr) residues in vegetable farmers' blood that indicate chronic exposure to pesticides. This study therefore investigated the level of organochlorine and pyrethroid pesticide residues in vegetable farmers' bloodin Southern Benin. During October 2017 and January 2018, blood samples were collected from 22 and 20 vegetable and non-vegetable farmers (control group) respectively. Solid Phase Extraction (SPE) was used to extract OC and Pyr residues from the blood samples. Residues in the blood were quantified using Gas Chromatography coupled with Mass Spectrometry (GC/MS). Data were analyzed with R Statistical Software version 3.4.3. Independencetest X^2 was used to establish the relationship between to belong vegetable farmer group and presence of pesticide residues in the blood. The difference are statistically significant when $p < 0.01$. Among the pesticide residues found in the blood of the farmers and the control group, the concentrations of cyfluthrin, cypermethrin, hexachlorocyclohexane (β-HCH) and β-endosulfan were significantly different ($p < 0.01$). Blood of the vegetable farmers was found to contained OC (β-HCH and β-endosulfan) and Pyr (cyfluthrin, cypermethrin) pesticide residues that they used against pests. These compounds may be responsible for different health problems observed among the vegetable farmers in Southern Benin.

Keywords Southern Benin; blood; vegetable farmers; pesticide; gas chromatography

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1. Introduction

Like other cities in sub-Saharan Africa, vegetable farming is well developed in Cotonou, the economic capital of Benin [1]. This sector of urban agriculture has provided economic and nutritional value for the population [2]. Consumption of vegetables is highly nutritious with low energy values needed for the effective functioning of the human body [3]. Unfortunately, the vegetables are attacked by pests (insect pests, molds and bacteria, viruses), leading to reduced yield and lowered nutritional value [4]. In a view to protect vegetable crops against pests and increase their yield, vegetable farmers use phytosanitary products including chemical pesticides [5].

Several insecticide families are used against insect pests which attack plants. These include organochlorines (OCs), organophosphorus (Op) and pyrethroids (Pyr) [6]. Due to the persistence and toxicity of OCs, many of their representative compounds have been removed from the pesticide market and are therefore banned from use [7]. Despite this, the studies of Azandjèmè et al., reported the presence of Dichlorodiphenyltrichloroethane (DDT) residues (OC) in the blood of cotton growers in Benin [8]. This study made an association between the presence of DDT and the occurrence of diabetes II in these cotton farmers [8].

Pyr and Op compounds are used as OC pesticides substitutes in vegetable farming in southern Benin [9,10] because they have low toxicity compared to OC [11,12]. In Benin, however, no study has examined the presence of these residues in the blood of the vegetable farmers who are exposed to them. Our previous study on the influence of pesticide residues on the health of these vegetable farmers reported adverse effects of pesticides on biochemical and hematological parameters of these vegetable farmers [13]. It is in this context that we initiated this study aimed at evaluating the level of pesticide residues in the blood of vegetable farmers in Southern Benin.

2. Material and Methods

2.1. Inclusion and Exclusion Criteria

Included in this study were vegetable farmers between 18 and 60 years old who must have used pesticide one month prior sample collection and gave consent. For the control group, non-vegetable farmers or people who did not work in the chemical industry and who had no history with chemicals and did not live close to the vegetable farming sites. Those excluded were individuals who are less than 18 years old, vegetable farmers who did not give their consent, who did not use pesticides one month prior this study, and individuals with debilitating medical conditions (e.g., diabetes, hypertension, renal failure, viral hepatitis); smokers and alcohol drinkers were also excluded in this study. A total of 22 vegetable farmers and 20 control individuals were recruited.

2.2. Blood Sampling

During October 2017 and January 2018 blood samples were collected from 22 vegetable farmers and 20 non vegetable farmers. Blood samples were taken from the farmers during agricultural activity while it was taken at any convenient time from the control group. Blood was taken aseptically into EDTA (ethylene diamine tetra acetic acid) tubes.

2.3. Chemical Standards

All glassware used for extraction and cleaning was thoroughly washed with water and detergent and then rinsed twice with distilled water. They were again rinsed with acetone and finally dried in an oven. HPLC grade acetonitrile for analysis of pesticide residues was obtained from Carlo Erba reagents SAS, Val de Reuil, France. HPLC grade methanol for analysis of pesticide residues was obtained from J.T. Baker Mallinckrodt Baler, Deventer-the Netherlands. Ammonium sulfate was obtained from Acrosof, New Jersey, USA. SPE oasis Prime cartridges HLB 1cc 30mg (reference 186008055) were obtained from Waters SAS, Saint-Quentin-en-Yvelines, France.

2.4. Pesticide Residues Extraction

Blood samples were collected from the participants and were centrifuged at 3000 rpm/min for 15 min, after which the sera were separated into Eppendorf tubes. Proteins contained in the sera were precipitated by adding 400 μL of ammonium sulfate 2% to 250 μL of plasma sample and vortexed for 10 s. Then, 750 μL of acetonitrile was added to the mixture and vortexed for 10 s and centrifuged (Thermo Scientific SL 16R) for 4000 rpm for 5 min. About 800 μL of the supernatant obtained was diluted with 2.2 mL of distilled water. 3 mL of diluted sample was used to load the SPE oasis Prime HLB 1cc 30mg cartridge (reference 186008055) and the cartridges rinsed with 1 mL of 95:5 v/v water/methanol mixture. Finally, 1 mL of 90:10 v/v acetonitrile/methanol was used to elute the cartridge. The extract obtained was concentrated under a nitrogen stream to a volume of 200 μL and then 3 μL of the extracts were injected into the GC/MS.

2.5. Gas Chromatograph---Mass Spectrometry Analysis

Gas chromatograph (Varian Saturn GC 2200, Agilent Technologies, Les Ulis, France) with a Mass Spectrometry (Varian CP-3800) detector equipped with autosampler (Varian CP 4800) injector was employed. A fused silica ZB-5MS capillary column (30 m $\times$ 0.25 mm $\times$ μm) was used with the following oven temperature program: initial temperature 70 °C, increased at 50 °C/min to 150 °C, ramp at 5 °C/min to 180 °C and finally increased at 1 °C/min to 183 °C and held for 6 min. Helium (99.99%) was used as a carrier gas (1.0 mL/MIN). A volume of 3.0 μL was injected in splitless mode. Mass spectrometer in electron impact mode (70 eV), scanning from m/z 50 to 650 was used in Specific Ion Storage (SIS) mode. MS workstation version 6.9.1 enabled data aquisition in single ion monitoring mode. The SIS mode was used to select specific ions in the trap. The ejection of the unwanted ions was performed by applying a multifrequency waveform, which includes those frequencies required to eject the unwanted ions and misses the frequencies corresponding to the stored ions. Specific ions and retention time of pesticides standard used in building the SIS method is presented in the Table 1. The pesticide residues in the blood samples was identified based on comparison of the measured relative retention times and specific ions of standards. The residue levels of OC and Pyr residues were quantitatively determined by the standard method using a peak area. Measurement was carried out within the linear range of the detector. The peak areas whose retention times coincide with the standards were extrapolated on their corresponding calibration curves to obtain the concentration. The quality was assured through the analysis of solvent blanks and procedure blanks. The method was optimized and validated using spiked (together) with the internal standard to evaluate the recovery of compounds. The recoveries of the standards ranged between 92.18% and 98.12% for all OC and Pyr pesticide residues. The limit of detection was 0.015 ppb.

Table 1. Standard of pesticide residues with their retention time and specifics ions.

Pesticide Residues	Reention Time	Specifics Ions of Pesticide Residues
Organochlorine residues		
β-Hexachlorocyclohexane	12.894	109.1-183-218.9
Aldrin	18.937	66.1-263.1-293
α-Endosulfan	25.290	195.2-241.2-269.1-338.8
Dieldrin	28.120	79.1-279.0-337.0
Dichlorodiphenyldichloroethylene	28.300	246.5-318.1
β-Endosulfan	32.111	195.2-267-338.9
Dichlorodiphenyldichloroethane	33.772	235
Endosulfan Sulfate	37.487	239.2-272.1-386.9-423.9
Dichlorodiphenyltrichloroethane	38.985	235.2
Pyrethroid residues		
λ cyhalothrin	45.541	141.2-181.2-208.1
Cyfluthrin	47.485	91.1-127.1-163.2-206.3
Cypermethrin	47.758	91.1-127.2-163.2-206.2-226.1
Fenvalerate	48.986	125.2-167.2-181.3-225.2-419.2
Deltamethrin	49.938	172.2-181-253.2

2.6. Statistical Analysis

Results obtained were analyzed using R Statistical Software version 3.4.3. An X^2 test was used to determine the link between presences of pesticides residues in the plasma and being a vegetable farmer. Anova was used to compare the mean of pesticide residues in the plasma of vegetable farmer and control group. The statistical significance for each analysis was considered at $p < 0.05$.

3. Results

The X^2 independence test showed that there was a relationship of dependence (x^2 = 14.91, dl = 9, Prob = 0.093) between vegetable farmers and having pesticide residues in their blood. Figure 1 shows the proportions of the respondents with traces of pesticide residues. The residues are present in the blood of all vegetable farmers but not in all the control groups.

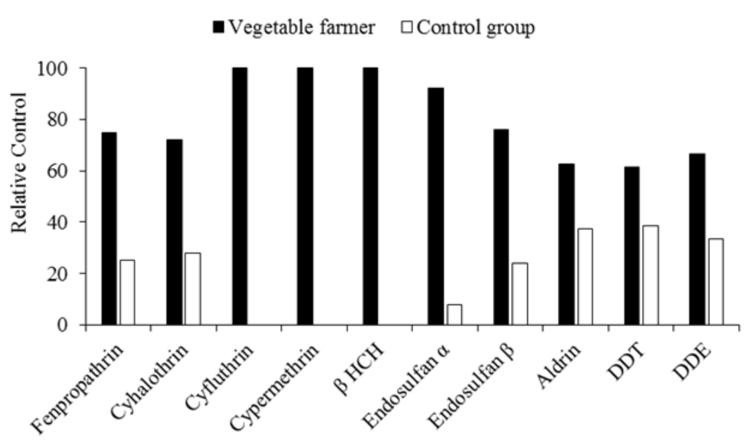

Figure 1. Pesticide residues found in the vegetable farmers and non-vegetable farmers' blood.

Table 2 shows the average concentrations of pesticides in the blood of the participants. The results indicates that the average concentrations of Cyfluthrin, Cypermethrin, β-HCH and Endosulfan β are statistically different ($p < 0.05$).

Table 2. Results of variance analysis on pesticide residues. Blod value: $p < 0.05$.

Pesticide Residues	dl	F Value	*p*
Cyfluthrin	1	4.18	**0.048**
Cyperméthrin	1	5.83	**0.020**
DDE	1	0.95	0.337
β HCH	1	17.96	**0.000**
α-Endosulfan	1	2.77	0.107
β-Endosulfan	1	7.65	**0.010**
Aldrin	1	-	-
Fenpropathrin	1	-	-
λCyhalothrin	1	-	-
DDT	1	-	-

Figure 2 shows the average pesticide residue concentrations in the two groups. Generally, the average concentrations of all the residues were higher in vegetable farmers compared to non-vegetable farmers.

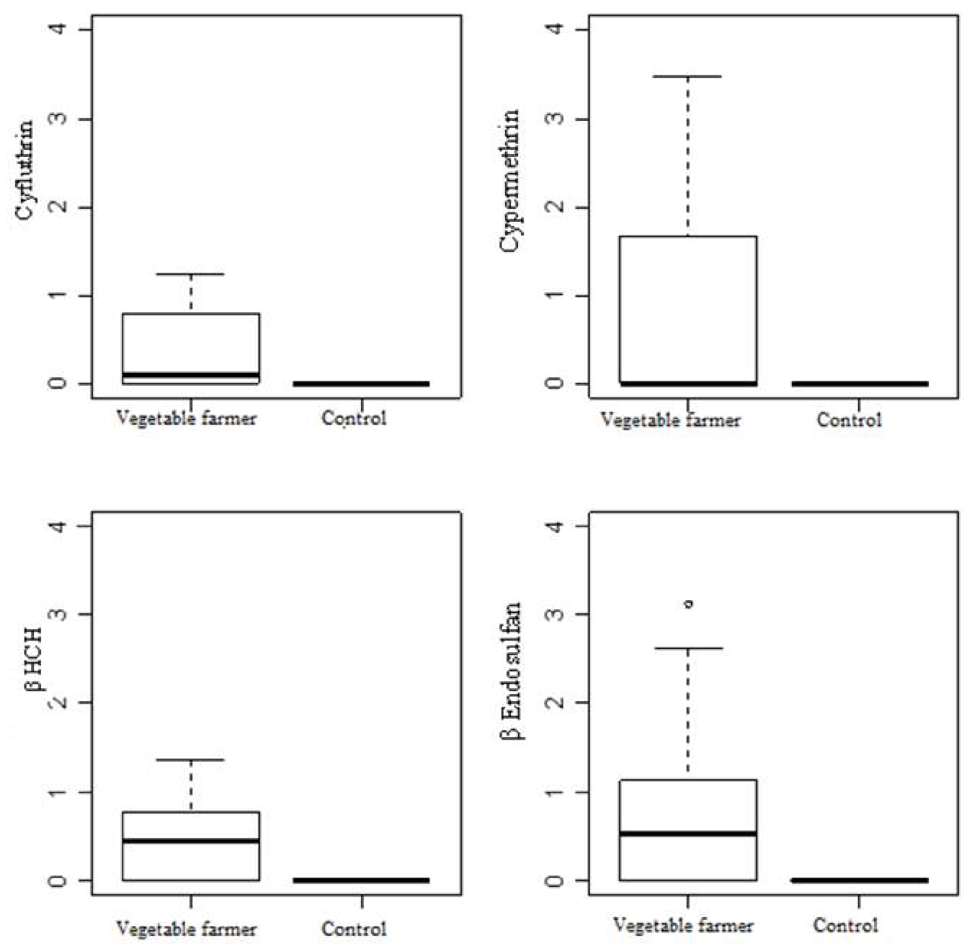

Figure 2. Level of Cyfluthrin, Cypermethrin, β-HCH and β-Endosulfan in vegetable farmers' and the control blood.

4. Discussion

The widespread use of pesticides is considered as leading cause of problems and deaths in many parts of the world. This is the case for farmers who use them to control pests. Although protective measures are recommended before handling pesticides, many farmers in developing countries such as Benin, do not obey these recommendations. This therefore exposes them to pesticide residues through various routes such as the eyes, nose and skin.

The organochlorides found in the blood of vegetable farmers in our study are β HCH, aldrin, α-endosulfans and β-endosulfans, DDT and its metabolite Dichlorodiphenyldichloroethylene (DDE). These residues found in the vegetable farmers' blood belong to the toxic Class III of Cramer [14].

Traces of aldrin, a highly toxic molecule for human classified as a carcinogen group 3 by the International Agency for Research on Cancer (IARC) and also a toxic molecule for the nervous system was found in the blood of study participants. This molecule was found in the blood samples of agricultural workers involved in pesticide activities, agricultural workers involved in pesticide spraying activities and people living far from agricultural fields in the Vehari district of Pakistan [15]. In the blood of these subjects, aldrin was found with a quantifiable concentration that averaged 0.92 $ng.mL^{-1}$ [15]. DDT and DDE were also found in trace amounts in the blood of vegetable farmers with the exception of one participant who had DDE at 0.83 $\mu g.mL^{-1}$. DDT and DDE are toxic molecules that get into the blood through inhalation, ingestion and absorption through the skin. DDT, an endocrine disruptor is a residue that is related to the occurrence of breast and uterine cancer, while DDE is recognized as a xenoestrogen mutagen. These two residues can bind to the endogenous receptor. They are also toxic to the olphatic and reproductive cells of humans. DDE has been detected in the blood of people living from the environs of Akumadan community which actively produced vegetableswith an average concentration of 380 $\mu g.kg^{-1}$ [16]. In addition to aldrin, DDT and DDE, alpha (α) and beta (β) endosulfan were also found in trace amounts at quantifiable concentrations. α-endosulfan is recognized as a mutagenic molecule and is very toxic to the endocrine system, whereas β-endosufan can penetrate the body causing headaches, dizziness, nausea and vomiting, as well as hyperexcitation of the muscles. These symptoms were found in our previous study on the health of vegetable farmers. A study conducted on the serum level of endosulfans resulting from occupational exposure to pesticide on farms show edendosulfan in high concentration with a mean of 60 ± 90 $\mu g.L^{-1}$ in the farmers' blood [17]. Our results show that the blood of Beninese vegetable farmers contains OC pesticides that are being used in farms. In addition, pyrethroid residues were detected.

Our study also reveals the presence of λ-cyhalothrin, fenpropathrin, cyfluthrine and cypermethrin in the blood of the study participants. These four detected pesticide residues all belong to Cramer Toxicity Class III. λ-cyhalothrin and fenpropathrin were detected in the blood of the vegetable farmers in low non-quantifiable concentrations (trace). These compounds are harmful when ingested, inhaled and in direct contact with the skin. When inhaled, both λ-cyhalothrin and fenpropathrin cause irritation of the respiratory tract. The λ-cyhalothrin is toxic to the thyroid glands and the nervous system as well as to olphatic cells. 3-phenoxybenzoic acid (3-PBA), a metabolite of λ-cyhalothin, was found in 42% of the blood samples of farmers in Fang District, Chiang Province, Northern Thailand, with a concentration range of 4.29 to 9.57 $ng.mL^{-1}$ [18]. cypermethrin and cyfluthrin were found at a high concentration in the blood of farmers in this study. The concentration of cypermethrin ranged from 1.68 to 4.82 $\mu g.mL^{-1}$, while that of cyfluthrin ranged from 0.13 to 6.12 $\mu g.mL^{-1}$. cyfluthrin is recognized as a toxic molecule for the liver and kidneys while cypermethrin is a possible carcinogen molecule. In the blood of Pakistani farmers, cypermethrin was found at a very high concentration of 32.40 $\mu g.mL^{-1}$ [19].

5. Conclusions

In conclusion, this study shows that Beninese vegetable farmers are exposed to the toxic pesticides that are being used in their farms. Among OC and Pyr residues, which are found in vegetable farmers' blood in Southern Benin, β endosulfan and β HCH (OC residues), cyfluthrine and fenpropathrin (Pyr residues) had the highest concentration. Our results show a chronic exposure to pesticide residues when vegetable farmers used them without protective equipment.

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