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# Distribution of Organochlorine Pesticides Residues in *Solanum macrocarpum* and *Lactuca sativa* Cultivated in South of Benin (Cotonou and Seme-Kpodji)

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**Abstract** Organochlorines pesticides (OCPs) which utilization is banned, affect nutrition quality of vegetables. Recent research shown their presence in different vegetables. Since last study carried out in 2007 about vegetables contamination by OCPs residues in Benin, none research is done about OCPs residues monitoring in vegetables. This study was conducted to monitor OCPs residues in two vegetables (*Solanum macrocarpum* L. and *Lactuca sativa* L.) collected from 4 farms sites in two communes (Cotonou and Seme-kpodji) of Benin. A total of 31 samples of *Solanum macrocarpum* L. and *Lactuca sativa* L. were collected from the study areas and analyzed for 13 OCPs residues, which can be grouped into DDTs, Aldrins, Endosulfans, HCHs and Methoxychlor. The data revealed that 100% of the vegetable samples were contaminated by OCPs residues. Aldrin, Dieldrin, Endrin, Endosulfan sulfate, pp'-DDE and pp'-DDT concentrations found were below the maximum residue limits (MRLs) set by European Union (EU) whereas 31.25%, 37.5%, 43.75% of β-HCH, α-Endosulfan, β-Endosulfan concentrations found in *Solanum macrocarpum* L. were above the EU MRLs adopted values. In *Lactuca sativa* L., the levels of β-HCH and β-Endosulfan were about 6.67% each and were above the EU (MRLs). Many of OCPs residues levels are below MRLs while three OCPs residues exceeded EU MLRs. This observation suggests that vegetables contamination is due to the persistence of OCPs residues in the soil where vegetables were cultivated. We therefore propose the monitoring of OCPs and other pesticide residues in vegetables cultivated in Benin to perform nutritional quality of vegetables.

***Keywords:*** *vegetables, organochlorine pesticides, Benin, health, environment*

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## 1. Introduction

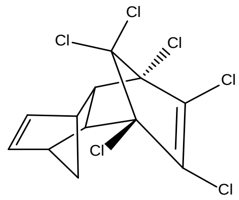
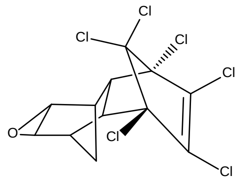
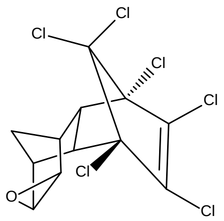
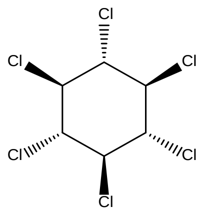
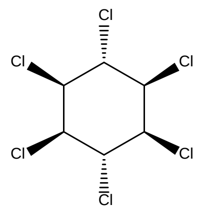
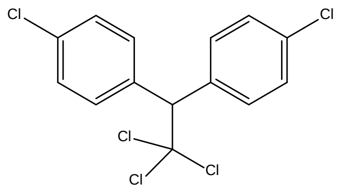
In southern of Benin, different types of vegetables are produced by the farmers [1]. Those vegetables could be attacked by pest because Benin is a West Africa country which has a Subequatorial climate, favorable to the proliferation of various pathogens including pests as insects, fungal species and other bacteria [2]. Those pests are responsible for diseases of vegetables [3]. Direct consequences are loss of crop yields and decline in cash income after vegetables selling. This situation is a major problem for vegetables farmers. To protect their vegetables, they use pesticides [4,5,6].

Pesticides are widely used on vegetables to control pests and diseases during farming, transportation, and storage. Pesticides are known to be the most important tool for the production of adequate food supply for an increasing world population and for the control of vector-borne diseases.

Organochlorine pesticides (OCPs) are the first chemical representatives of insecticides used to control pests insect. This is the case of DDT and another active ingredient (Endosulfan, Toxaphene, Chlordane, Heptachlor and Dieldrin, etc) [7]. DDT and its metabolites are harmful to human health [8]. Exposure to OCPs at chronic, acute or lethal dose, is associated with diseases that can lead to death. Several studies and surveys on the impact of OCPs on health and the environment have linked OCPs to various pathologies including endocrine disruption, dysregulation of reproductive function and immune system, neurobehavioral disorders [9,10,11,12,13]. The major characteristics of OCPs are their resistance to biological, photolytic and chemical degradation [14,15,16]. They also have low solubility in water but high solubility in fat, so it is the main reasons which could explain their bioaccumulation in adipose tissues [17,18]. These characteristics added to their semi-volatility, enable them to travel long distances in the atmosphere before being deposited [19,20]. Most of them were banned (Annex A POPs) or at least severely restricted (Annex B POPs) [21]. Table 1 shows the structure of those molecules and their half-life in soils.

Nowadays, OCPs residues are found in vegetables [22,23]. Reference [1] research in south of Benin, shown the presence of organochlorine (DDT, Endrine, Heptachlore, Aldrine and dieldrine) in two local vegetables (*Solanum macrocarpum* L. and *Amaranthus cruentus* L.). Since that research, there isn't any study about vegetable's contamination by OCPs. It is in this context that we conducted this research to investigate the distribution of organochlorine pesticides in *Solanum macrocarpum* L. and *Lactuca sativa* L.

**Table 1. Chemical structures of organochlorine pesticides struck out and restricted by Stockholm convention on Persistent Organic Pollutants (POPs)**

| Organochloride residues | Half-Life | Structure |
|---|---|---|
| **Elimination** | | |
| **Aldrin** | 20-100 days |  |
| **Dieldrin** | 5 years |  |
| **Endrin** | Up to 12 years |  |
| **β Hexachlorohexane** | 7-8 years |  |
| **γ Hexachlorohexane** | Up to 3 years |  |
| **Restriction** | | |
| **Dichloropiphenyltrichloroethane** | 2 – 15 years |  |

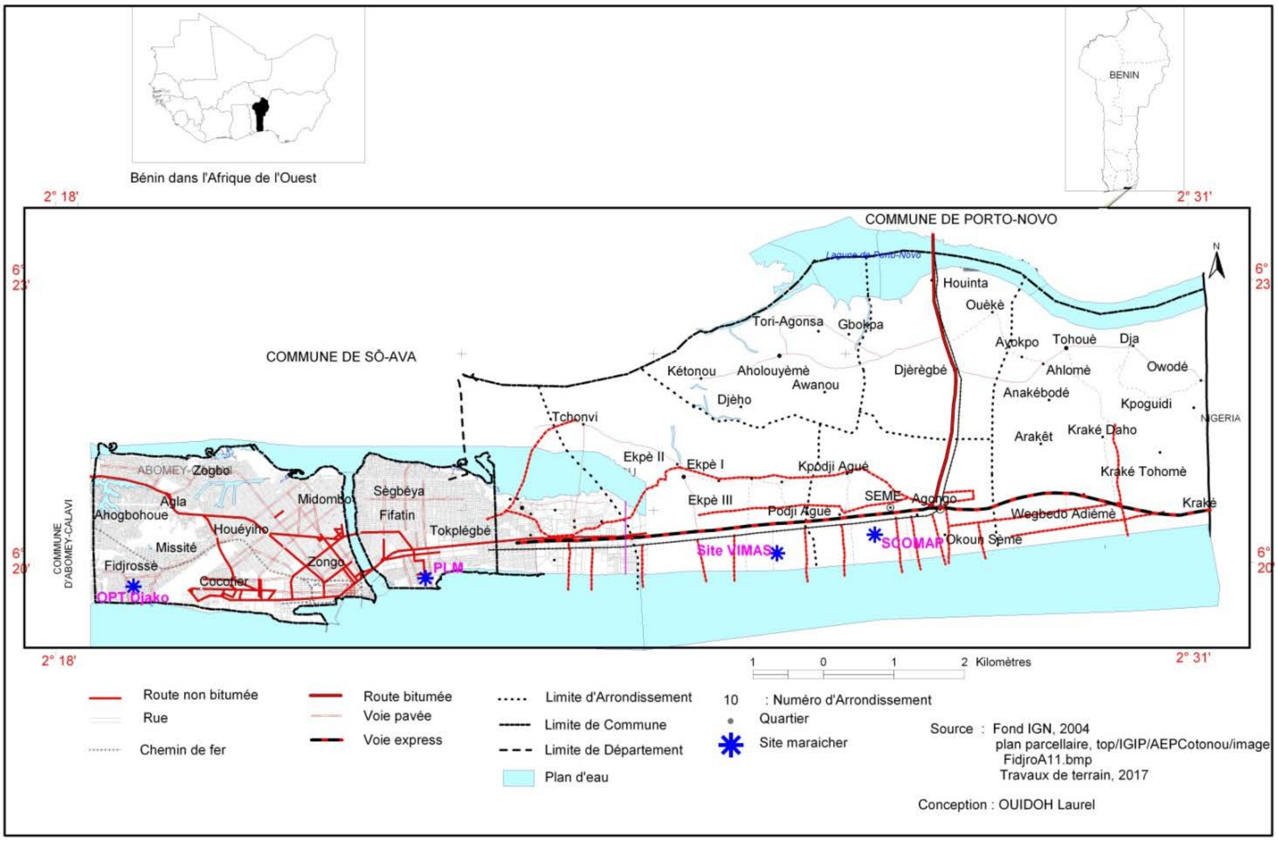



**Figure 1**. Map of study area with sampling points

# 2. Materiel and Methods

## 2.1. Study Areas

Sample collection was done during the great rainy season of 2017 from March to July. The samples were collected on two vegetables farms sites located in Cotonou, intra-urban area and two vegetables farms sites located in Seme-kpodji, Suburban area. (Figure 1)

## 2.2. Sampling

A total of 31 samples of vegetables were collected from the study areas including 15 samples of *Lactuca sativa* L. and 16 *Solanum macrocarpum* L. These vegetables were chosen because they are very common in Beninese diet.

The sampling plan consisted to delimit a 10 x 10 m plot in the middle of the field of each farmer. Five primary samples were chosen along the diagonals. These samples were roughly cut and mixed in situ to obtain a final sample of 750 g. Each sample was packed in aluminum foil, plastic bag and conserved in ice box. The sample was sent to the laboratory and it was stored at -20°C until the moment of the analysis.

## 2.3. Extraction and Clean-up

A sample of 15±0.1g of each vegetable was weighed into a 50mL centrifuge tube, 15 mL of acetonitrile (ACN) (HPLC grade) were added and the tube was closed and shaken vigorously by shaker for 1 min. After shaking a mixture of 4 g magnesium sulphate, 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogenocitrate sesquihydrate were added. The tube was closed and immediately shaken vigorously by shaker for 1 min and centrifuged for 10 min at 3000 rpm.

An aliquot (6 mL) of the extract was transferred into centrifugation tube which contains following sorbents: 150 mg of anhydrous magnesium sulphate, 50 mg of PSA, 50 mg of black graphite Carbon and 50 mg of adsorbent C18 for purification. Before shaken we added to the tube formic acid at 10µL per mL of extract (1%) and shaken vigorously for 1 min and centrifuged for 10 min at 3000 rpm. After, 4 mL of the extract cleaned was concentrated in dessicator witch contains silica gel activated by heating in oven at 105°C. The residue was recoved with 1 mL of the mixture of formic acid in ACN (1%) and introduced in vial.

## 2.4. Gas Chromatograph-ECD

Measurements were carried out on Gas Chromatograph (GC) Varian CP-ECD with a CombiPAL Autosampler. GC was equipped with an Electron Capture Detector. The chromatographic separation was done on capillary column with 30 m + 10m EZ x 0,25 mm internal diameter fused silica capillary coated with VF-5ms (0.25 µm film) from Varian Inc or equivalent.

The oven temperature program was set as follows: Initial temperature was set at 70°C for 2 min and ramped at 25°C/min to 180°C for 1 min and then 300°C at a rate of 5°C/min a total run time of 30 min. The injector setting is a pulsed splitless mode with a temperature of 270°C.

The detector temperature was 300°C in "constant flow 1ml/min of Nitrogen gas) and made up at 29 ml/min.

The residue of pesticide was identified based on comparison of the measured relative retention times to those of known standards. The residue levels of organochlorine pesticides were quantitatively determined by the external standard method using 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 (Table 2).

**Table 2. Organochlorines pesticides analyzed with their retention time**

| Pesticides residues | Ret time |
|---|---|
| β HCH | 12.4 |
| Δ HCH | 13.24 |
| γ HCH | 17.43 |
| Methoxychlor | 23.17 |
| Aldrin | 15.28 |
| Dieldrin | 18.71 |
| Endrin | 19.48 |
| α Endosulfan | 17.81 |
| β Endosulfan | 19.95 |
| Endosulfan Sulfate | 21.24 |
| p p'DDE | 18.49 |
| p p'DDD | 20.10 |
| p p' DDT | 21.35 |

## 2.5. Quality Control

Our samples were analyzed at Ghana Standards Authority which have accreditation in chemical analysis of fruits and vegetable 'terms of DIN EN ISO/ ICE 17025;2005'. Their registration number of the certificate is D_PL_152009_01-02. So the quality of organochlorine pesticides was assured through the analysis of solvent blanks, procedure blanks. The method was optimized and validated using spiked together with the internal standard to evaluate the recovery of compounds. The recoveries of internal standards ranged between 71.4% and 104% for all the organochlorine pesticides. The limit of detection was 5 ppb.

## 2.6. Statistical Analysis

Results obtained are expressed in mean ± standard deviation of the mean. Statistical analysis were performed by Student test unmatched to compare samples mean. The second test was Chi-square and it was used to determine the difference between the number of vegetables samples which the level in OCPs residues are above the MRLs. The statistical significance for each analysis was considered at $P < 0.05$. MedCalc Statistical Software version 15.0 was used for the statistical analysis.

# 3. Results

The mean total concentration of OCPs (0.016±0.045) found in *Solanum macrocarpum* L. is higher than the total concentration (0.003±0.018) found in *Lactuca sativa* L., as indicated in Figure 2. But the difference isn't not statistically significant ($p > 0.05$).

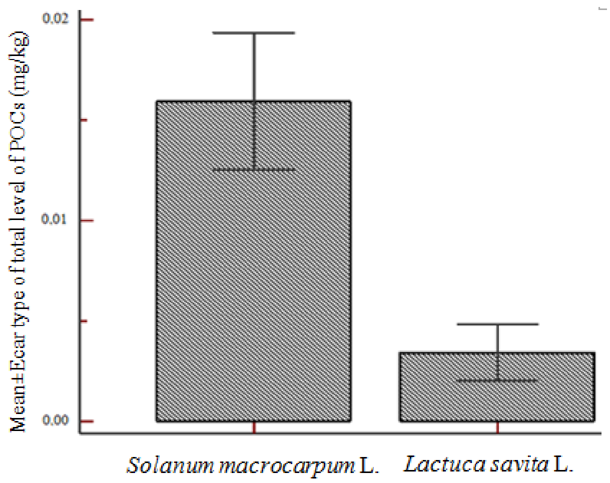



**Figure 2.** Total level of Organochlorine residues in *Solanum macrocarpum* L. and *Lactuca savita* L.

Our research about Hexachlorohexane (HCH), Aldrin, Endosulfan, DDT, Methoxychlor and related isomers (γ-HCH, β-HCH, Δ-HCH, β-Endosulfan, Endosulfan-sulfate, Dieldrin, Endrin and pp'-DDE) shown their presence in all vegetables samples except Δ-HCH and Methoxychlor.

Detection of the frequency of individual organochlorines residues analyzed in *Solanum macrocarpum* L. and *Lactuca savita* L. samples from the differents sites is indicated in Figure 3. Among HCH isomers detected, β-HCH frequency was 73.33% in *Lactuca savita* L. and 73.50% *Solanum macrocarpum* L. γ-HCH wasn't detected in *Lactuca sativa* L. while it frequency in *Solanum macrocarpum* L. was 37.5%.

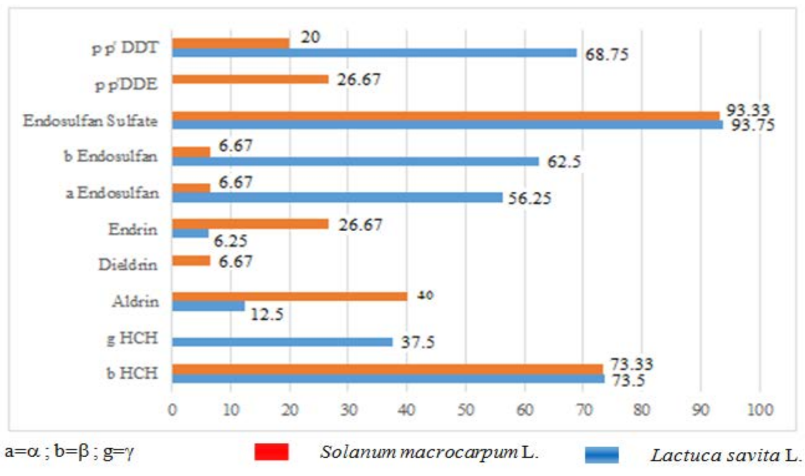



**Figure 3.** Organochlorines Pesticide residues frequency in tested vegetables

Related frequency of Aldrin isomers found in *Lactuca sativa* L. are higher than *Solanum macrocarpum* L. Aldrin was found in 40% of *Lactuca sativa* L. against 12.5% in *Solanum macrocarpum* L. 26.67% of *Lactuca sativa* L. contained Endrin against 6.25% of *Solanum macrocarpum* L. Dieldrin wasn't detected in *Solanum macrocarpum* L. while it was found in 6.67% of *Lactuca sativa* L. samples which were analyzed.

Out of Endosulfan sulfate which it frequency in *Lactuca sativa* L. and *Solanum macrocarpum* L. are respectively 93.33% and 93.75%. α and β-Endosulfan frenquency are higher (56.25% and 62.5%) in *Solanum macrocarpum* L. than *Lactuca sativa* L. (6,67% and each).

pp'-DDT was found in 68.75 % of *Solanum macrocarpum* L. against 20% of *Lactuca sativa* L. Isomer pp'-DDE wasn't present in *Solanum macrocarpum* L., but it was found in 26.67% of *Lactuca savita* L.

Table 3 shows minimum, maximum, mean ± standard deviation and total concentration of OCPs residues isomers in *Solanum macrocarpum* L and *Lactuca sativa* L.

Among HCHs isomers detected in the samples, β HCH concentration (0.122 mg/kg) was the high level detected in *Solanum macrocarpum* L. Totat concentration of HCHs found in *Solanum macrocarpum* L. was higher six times than HCHs concentration found in *Lactuca sativa* L.

Among Endosulfan isomers detected in the samples, β-Endosulfan concentration (0.279 mg/kg) was the high level detected in *Solanum macrocarpum* L. Total concentration of Endosulfans quantified in *Solanum macrocarpum* L. was higher seven times than Endosulfans level in *Lactuca sativa* L.

Between DDT and it metabolite pp'-DDE detected in the samples, pp'-DDE concentration (0.092 mg/kg) was the high concentration and it was detected in *Lactuca sativa* L. Total concentration of DDT and DDE found in *Lactuca sativa* L. was the double of their concentration found in *Solanum macrocarpum* L.

In descending orders, total concentration of OCPs found in *Solanum macrocarpum* L. was ΣEndosulfans > ΣHCHs>ΣDDT>Σaldrin while in *Lactuca sativa* L., it was ΣDDT>ΣEndosulfans>Σaldrins=ΣHCHs.

**Table 3. Organochlorines Pesticides residues concentrations in the tested vegetables (mg/kg)**

| **Organochlorine Pesticides Residues** | ***Solanum macrocarpum L*** | | ***Lactuca Savita L*** | |
|---|---|---|---|---|
| | **Range** | **Mean±SD** | **Range** | **Mean±SD** |
| β HCH | 0.001-0.122 | $24.2x10^{-3}±40.90x10^{-3}$ | 0.001-0.015 | $4.40x10^{-3}±4.66x10^{-3}$ |
| γ HCH | 0.001-0.008 | $1.69x10^{-3}±2.73x10^{-3}$ | ND | -- |
| **ΣHCHs** | **0.415** | | **0.066** | |
| Aldrin | 0.001-0.002 | $0.19x10^{-3}±0.54x10^{-3}$ | 0.001-0.019 | $2.53x10^{-3}±5.18x10^{-3}$ |
| Dieldrin | ND-0.002 | -- | ND-0.006 | $0.40x10^{-3}±1.55x10^{-3}$ |
| Endrin | ND-0.002 | $0.13x10^{-3}±0.50x10^{-3}$ | 0.004-0.008 | $1.47x10^{-3}±2.67x10^{-3}$ |
| **Σaldrins** | **0.005** | | **0.066** | |
| α Endosulfan | 0.008-0.227 | $56.4x10^{-3}±76.70x10^{-3}$ | ND-0.002 | $0.13x10^{-3}±0.52x10^{-3}$ |
| β Endosulfan | 0.005-0.279 | $75.4x10^{-3}±95.70x10^{-3}$ | ND-0.213 | $14.2x10^{-3}±55x10^{-3}$ |
| Endosulfan S | 0.005-0.031 | $12.8x10^{-3}±8.03x10^{-3}$ | 0.005-0.015 | $6.33x10^{-3}±3.04x10^{-3}$ |
| **Σ Endosulfans** | **2.313** | | **0.31** | |
| p p'DDE | ND | -- | 0.004-0.092 | $8.00x10^{-3}±23.8x10^{-3}$ |
| p p' DDT | 0.001-0.013 | $2.94x10^{-3}±4.34x10^{-3}$ | 0.002-0.003 | $0.47x10^{-3}±0.99x10^{-3}$ |
| **Σ DDT** | **0.047** | | **0.127** | |

ND : No Detected.

**Table 4. Maximum residue limits (MRLs) of OCPs in vegetables and its percentage above MRLs**

| Organochlorine Pesticides Residues | *Solanum macrocarpum L* (n=16) | | *Lactuca savita L* (n=15) | | $X^2$ | *p-value* |
|---|---|---|---|---|---|---|
| | LMR UE (mg/kg) | % Residues level > LMR UE | LMR UE (mg/kg) | %Residues level > LMR UE | | |
| β HCH | 0.01 | 31.25 | 0.01 | 6.67 | 1.629 | 0.20 |
| γHCH | -- | -- | -- | -- | -- | -- |
| Aldrin | 0.01 | 00 | 0.01 | 00 | -- | -- |
| Dieldrin | 0.01 | 00 | 0.01 | 00 | -- | -- |
| Endrin | 0.01 | 00 | 0.01 | 00 | -- | -- |
| α Endosulfan | 0.05 | 37.5 | 0.05 | 00 | 4.779 | 0.03 |
| β Endosulfan | 0.05 | 43.75 | 0.05 | 6.67 | 3.792 | 0.05 |
| Endosulfan Sulfate | 0.05 | 00 | 0.05 | 00 | -- | -- |
| ΣEndosulfans | 0.05 | 50 | 0.05 | 6.67 | 5.109 | 0.02 |
| p p'DDE | 0.05 | 00 | -- | -- | -- | -- |
| p p' DDT | 0.05 | 00 | 0.05 | 00 | -- | -- |
| ΣDDTs | 0.05 | 00 | 0.05 | 6.67 | | |

Table 4 presents the Maximal Residue Level and the percentage of the residues which the level are exceeded the limite.

Among OCPs residues analyzed, only three residues level were above Maximum Residue Level of European Union [24]. These residues were β HCH, α Endosulfan and β Endosulfan. Most of *Solanum macrocarpum* L. samples presented a high level of β HCH, α Endosulfan and β Endosulfan than samples of *Lactuca sativa* L.

It's very important to note that among OCPs residues analyzed, HCH, Endosulfan and DDT haven't got MRLs for *Solanum macrocarpum* L. and *Lactuca sativa* L. Aldrin and Dieldrin which have got FOA/WHO MRLs were detected in the vegetables samples at concentration below FOA/WHO MRLs [25].

## 4. Discussion

Despite the fact that OCPs have been banned in developing countries like Benin, this study conducted about distribution of OCPs residues in vegetables has shown their presence.

Reference [26,27], also found α Endosulfan, β Endosulfan, γ HCH, pp' DDT, pp' DDE, Dieldrin and Aldrin in different vegetables such as onion, lettuce, tomato and cabbage from Nigeria and Ghana. In another countries such as China and India, DDT, HCH, DDE, α Endosulfan and β Endosulfan were detected in vegetables [28,29].

Total concentration of HCHs in lettuce of China province (Deyang and Tianjin) and urban market's of Ghana were lower than HCHs concentration in our research [28,29,30].

The level of Aldrin concentration found in lettuce from Ghana was lower than Aldrin level in our study [31]. In the same study, Dieldrin and Endrin concentration present in lettuce was lower than the limit of detection [31]. This finding is contrary with our results. Dieldrin was also detected in lettuce from Wako (Nigeria town) and its mean was 0.008 mg/kg [32].

Different isomers of Endosulfan (α and β) were also found in vegetables from different countries of Africa such as Nigeria and Tanzania. The concentrations of Endosulfan was 44.91 µg/kg for Nigeria's samples; α Endosulfan and β Endosulfan level were 0.6 mg/kg and 0.2 mg/kg respectively in vegetables samples from Tanzania. Endosulfan concentration we found in *Solanum macrocarpum* L. was higher than it concentration in Nigeria's vegetables. α Endosulfan and β Endosulfan found in Tanzania's vegetables were lower than the concentration we found in *Lactuca sativa* L. [19-33].

A similar research conducted in West Bengal, India, to access the level of organochlorine pesticides residues in vegetables revealed that, the concentration of ΣOCPs was ranged between, <0.01–65.07µg/kg with average of 9.67±2.34µg/kg (wet/wet.). The concentration of ΣDDT was 3.49±0.93µg/kg (wet/wet) which was lower than our concentration found in vegetables [34].

Among OCPs residues analyzed, only three residues level were above Maximum Residue Level of European Union. These residues are β HCH, α Endosulfan and β Endosulfan. Most of *Solanum macrocarpum* L. samples presented a high level of β HCH, α Endosulfan and β Endosulfan than samples of *Lactuca sativa* L.

Contrary to our results, Reference [1] found in *Solanum macrocarpum* L., Endosufan and γ HCH residues at level below Maximum Level Residue. In that study, Aldrin, Dieldrin, Endrin and DDT concentration found were above Maximum Level Residue. This finding is supporting by [33] who detected in vegetables samples from two agriculture sites of Nigeria, ΣDDT, Aldrin and Dieldin level above MRL. In lettuce samples of Togo, HCH, DDT, Aldrin, Dieldrin and Endrin were detected at levels below EU MRL [23].

[35] The litterature revealed that the HCH level was above the MRL in *Solanum licopersicum* from farm site of Nigeria. Reference [32,33,34,35,36], detected respectively Aldrin in apple, Dieldrin in lettuce and Aldrin in cabbage with level below the MRL.

The predominance of DDT found in vegetable samples compared to its metabolites showed that DDT had been used recently by the vegetable famers. But Aldrin, Endosulfan found indicated that they were used only in past time and no recent input into environment because the ratio of ΣEndosulfans and ΣAldrins on their metabolites are lower than 0.5.

Endosulfan used against *Helicoverpa armigera* was prohibited in developing country like Benin [37]. The level of that molecule in *Solanum macrocarpum* L. suggests that the sanitary quality of that vegetable is affected and posed a major problem of public health. Indeed, 105 cases of poisoning due to this molecule were registered in Benin between 2007-2008 [38].

## 5. Conclusion

The use of the QuEChERS method and the GC-ECD enabled us to research and quantify the OCPs in vegetables grown in market gardening in southern Benin. From our results, it appears that, despite their long-standing ban, OCPs continue to be detected in vegetable crops. The reason for the presence of OCPs in vegetables in Benin can be explained by their massive use in the past in agriculture and their persistence. About this situation, awareness and coercive measures are needed to control OCPs in all vegetables as phytosanitary prohibited products may be used in the sub-region in general and in Benin in particular. we suggest the monitoring of OCPs and others pesticides for consumer health protection.

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