Assessment of vegetables and the environment contamination in market-gardening in southern-Benin Using Aedes aegypti larvae to assess pesticide contamination of soil, groundwater and vegetables

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ABSTRACT

In Benin the use of synthetic pesticides in vegetable production poses a risk to the environment and human health. The use of synthetic pesticides in Benin urban and periurban vegetable growing presents environmental and health risks due to farmers' practices. Vegetables, water and soil quality assessment is very important for monitoring and mitigation of these risks. The evaluation of degree of pesticide contamination of vegetables and agricultural environment is often made using expensive methods. It is a challenge for research in so-called developing countries to develop less expensive tools for pesticide risks assessment and monitoring. The aim of this study was to assess the potential of using Aedes aegypti larvae as a bio-indicator to measure the pesticide contamination of the agricultural environment and soil, groundwater and vegetables growing in southern Benin using Aedes aegypti larvae as bio-indicator. Samples of vegetables just before harvest, groundwater, and soils samples from three production sites and vegetables sold in Cotonou's samples from markets were collected from March to August 2011. Ethanol extracts of these samples collected were tested on first stage larvae of Aedes aegypti. The method made it possible to detect residues of chlorpyrifos-ethyl and deltamethrin in cabbage respectively until 4 and 8 days respectively after treatment with the recommended doses for crop protection on culture respectively. This method proved inappropriate to measure pesticides residues in market-gardening soils, but to suspect presence of pesticides residues because some of these soils already contain some amounts of nitrite, nitrate and phosphate, coming from the decomposition of fertilizers which are poisonous for the first stage larvae of Aedes aegypti. Generally, Overall, the results revealed the presence of small amounts of pesticides residues in 12.5% of the vegetables.

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collected from markets available for sale. Pesticide residues were also detected in 30.0% of vegetables collected just before harvest. No residues of pesticides were not detected in groundwater samples taken from cropping areas in vegetable growing areas production sites. The method made it possible to detect residues of chlorpyrifos-ethyl and deltamethrin in cabbage respectively 4 to 8 days respectively after treatment with the recommended doses on culture. But this method proves inappropriate to seek the presence of pesticide residues in market-gardening soils because they already contain some amounts of nitrite, nitrate and phosphate, coming from the decomposition of manures which are poisonous for the first-stage larvae of Aedes aegypti. This bio-indicator used to evaluate the pesticide contamination of vegetables by the pesticides was sensitive to organophosphate proved to be more sensitive to pyrethroid, and was inexpensive and easy to use. First stage larvae of Aedes aegypti could be used as a bio-indicator to characterize and monitor risk of pesticide contamination of vegetables knowing that pyrethroids and organophosphates are frequently used in southern Benin vegetable farms to control pests. It could also be used for a monitoring program before running a more thorough chemical analysis to identify and quantify the pesticide molecules present in samples—chemical analysis like chromatography and mass spectrometry methods which would then be used for identifying and quantifying the molecules present in vegetables, groundwater and soils.

**Keywords:** pesticides, vegetable contamination, environment, bio-indicator, Southern-Benin

1. INTRODUCTION

The market-gardening production of vegetables constitutes an important branch part of urban and peri-urban agriculture. It contributes to food safety and by ensuring sufficient food availability for the population. Vegetables constitute an important source of proteins, vitamins and trace elements and are widely recognized for their contribution to human health benefits (Chu, Y.F. *et al.*, 2002; Singh, J. *et al.*, 2007). constitute an important source of plant protein, vitamins and trace elements for human consumption (Farnham, M.W., 2000; Caussé, M. *et al.*, 2003; Santos, C.A.F., Simon, P.W., 2006). The market-gardening production of vegetables provides income to a great number of individuals reducing the level of unemployment and it contributes to the creation of more than 600 000 direct jobs in Benin (PADAP, 2003). The production is ensured by small-scale producers organized in the big cities or in or on the peripheries of the big cities and generally occupying swamps or marshes areas for vegetable growing (Tokannou, R., Quenum, R., 2007). Although this activity presents a significant economic interest, it also has some harmful effects on the environment, particularly the problems of agricultural irrigation, reduction of the biodiversity (Pazou, E.Y.A. *et al.*, 2006a, 2006b) and on human health issues related to the use of the chemical pesticides (Samborn, M. *et al.*, 2004; Pazou, E.Y.A. *et al.*, 2006a, 2006b; Willaim, S., Garba, M.H., 2011; Pretty, J. *et al.*, 2011; Tomenson, J.A., Matthews, G.A., 2009) related to chemical pesticide use. The more toxic pesticides intended for use on cotton fields are often used for diverted towards the vegetable crops protection (Akogbéto, M. *et al.*, 2005; Ahouangninou, C. *et al.*, 2011). Similarly producers do not always respect the quantities guidelines and the preharvest intervals recommended (Rosendahl, I. *et al.*, 2009; Thiam, M., Touni, E., 2009). Lund, T. *et al.* (2010) reported that surveyed participants in Farmer Field School (FFS) approach did not adopt a complete package of Integrated Pest Management (IPM) tools and concepts. These farmers practices could have consequences on the chemical quality of the vegetables produced and thus generate health issues for consumers. More information on exposure to pesticides is needed (Probst, L. *et al.*, 2012a).

Thus vegetables, water and soil quality assessment is important for monitoring and mitigation of pesticide risks (Osman, K.A. *et al.*, 2010). To assess pesticide contamination in
vegetables, foods, groundwater and soils, conventional techniques like chromatography with mass spectrometry are frequently used (López-Blanco, M.C. et al., 2002; Rial Otero, R. et al., 2002, 2003; González-Rodriguez, R.M. et al., 2008; Kin, C.M. et al., 2008; Osman, K.A. et al., 2010; Lopez-Fernandez, O. et al., 2012). These conventional techniques are costly and expensive in developing countries. It is a challenge for research in developing countries to develop less expensive tools for pesticide risks monitoring-like bio-indicators. Bio-indicators are living thing (animals, plants and microorganisms) used to monitor environmental health. They can be organism or biological response that reveals the presence of pollutants by the occurrence of typical symptoms or measurable response, and are therefore more qualitative. Environment quality assessment using bio-indicators or ecotoxicological approach were reported in literature (Markwiese, J.T. et al., 2001; Cooman, K. et al., 2005; Deardorff, A.D., Stark, J.D., 2009; Palma, P. et al., 2010). The aim of this study was to assess the potential of using *Aedes aegypti* larvae as a bio-indicator to measure pesticide contamination of soil, groundwater and vegetables growing in southern Benin using *Aedes aegypti* larvae as bio-indicator. So to evaluate the impact of these practices on the quality of vegetables produced and on agricultural environment this study was carried out. The aim of the study is thus to assess the contamination of vegetables and the environment derived from vegetable growers practices in southern Benin using a bio-indicator.

2. MATERIAL AND METHODS

2.1 Sampling

To assess the potential of using *Aedes aegypti* larvae as a bio-indicator of pesticide contamination, we collected (1) cabbage and soil samples from trial plots, (2) vegetable samples from farms and markets, and (3) groundwater and soil samples from farming sites. Twelve samples of cabbages and soils were taken on six plots treated with deltamethrin (PLAN-D25EC) or chlorpyrifos-ethyl (PYRIFORCE 480EC) at a doses of 25 g and 480 g of active ingredients per hectare respectively in experiment field, and this to evaluate the evolution of the pesticides residues content. Deltamethrin and chlorpyrifos-ethyl are commonly used pesticides in Southern Benin. No manure was added during the study period to the sampled plots.

From March to August 2011, 20 vegetables samples were collected just before harvest on the market-gardening sites of Houéyiho, Sémé-kpodji, Ouidah and on the Cotonou's markets. Vegetables farms selected were already involved in the project “Ecosystemic Approach for Sustainable Vegetable Production Project” (EASVP) led by Cotonou's APPLIED BIOMEDICAL SCIENCE INSTITUTE (ISBA) and Hortsys Unit of INTERNATIONAL COOPERATION CENTER IN AGRONOMIC RESEARCH FOR DEVELOPMENT CIRAD. During sampling period, farmers received directive to inform us before harvesting in order to collect vegetable samples. Overall, 15 kinds of vegetables were sampled. They were the most commonly found species in the studies areas (C. Ahouangninou, ISBA, Unpublished results). Moreover, Overall 20 were taken just before harvest and 40 vegetable samples were collected in six markets of Cotonou and its peripheries. These markets included in the study and vendors were selected with random method. Samples were packed and sent to laboratory of MIVEGEC unit of the RESEARCH INSTITUTE FOR DEVELOPMENT (IRD) associated to the ENTOMOLOGICAL RESEARCH CENTER OF COTONOU (CREC) for toxicological analysis. Then, twelve samples of cabbages and soils were taken on six plots treated with deltamethrin (PLAN-D25EC) or chlorpyrifos-ethyl (PYRIFORCE 480EC) respectively at a doses of 25 g and 480 g of active ingredients per hectare in an exploitation respectively, and this to evaluate the evolution trend of the pesticides residues. In fact, these pesticides were used by several farmers during study period. No manure was added during the study period to the sampled plots.

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Finally fifteen samples of soils and groundwater were taken on the various market-gardening areas and sent to LABORATORY OF WATER AND FOOD QUALITY CONTROL of basic hygiene Direction of Cotonou in order to determine their concentrations in nitrates, nitrites and phosphates. Indeed, we suppose that fertilizers used by farmers could play a part-role in Aedes aegypti larvae mortality, thus influencing the reliability of the bio-indicator. Part of these soils and groundwater samples was sent to the laboratory of MIVEGEC unit of the RESEARCH INSTITUTE FOR DEVELOPMENT (IRD), to estimate the concentration of toxic residual expressed in equivalent-deltamethrin and equivalent-chlorpyrifos-ethyl.

2.2 Samples analysis

2.2.1 Pesticides analysis

Pesticide residues analysis was made using mosquito larvae taken as biological indicator of detection. The method of pesticides residues detection using a biological indicator (first stage larvae of Aedes aegypti) was first described by Martin, T. et al. (2007) to quantify pyrethroids on the treated mosquito nets impregnated and used by Ahouangninou, C. et al. (2012) to detect pesticides residues presence in vegetable before harvest in rural areas in Benin. This method does not aim to identify all the molecules present, but to compare the importance of the toxic answer observed on Aedes aegypti larvae with to references molecules which will be called “equivalent-deltamethrin” and “equivalent-chlorpyrifos-ethyl”. The insecticide deltamethrin was used because of its strong toxicity (LC50 of 0.4 µg/L) on Aedes aegypti larvae S-Be from north Benin (Ahouangninou, C. et al., 2012), but also because it belongs to the family of pyrethroid frequently used in vegetable protection in Benin. Chlorpyrifos-ethyl was used because it was used by most farmers during study period.

For each sample, 0.25 g of vegetables or 50 cm3 of soils that were weighed and put in a tube in which respectively 10 ml and 100 ml of ethanol were added respectively in order to extract the pesticides residues in 24 hours. After 24 hours, all pesticide residues contained in samples were extracted with ethanol. The use of ethanol as solvent to extract pesticide in vegetables was also reported by Wan, H.B. et al. (1994). A volume of 0.1 ml of extract was added to the contents of polystyrene goblet containing 9.9 ml of water with 20 first stage larvae of Aedes aegypti (three repetitions for each sample). The counting of mortality was made 24 hours after application. The test was taken again with two dilutions 1/10 and 1/100 in the event of total mortality. Natural mortality was taken into account by tests with distilled water containing ethanol 1%. A commercial formulation of PLAN-D25EC made up of 25 g/l of deltamethrin was used to establish the calibration line on Aedes aegypti larvae. A dilution in 1/400000 with distilled water was carried out and a series of ten successive dilutions were made to have a mortality ranging between 1% and 99%. The residual contents of pesticides present in vegetable leaves were evaluated in nanogram per gram of equivalent-deltamethrin starting from the calibration curve. A commercial formulation of PYRIFORCE 480EC made up of 480 g/l of chlorpyrifos-ethyl was also used to establish the calibration line on the larvae of Aedes aegypti. A dilution in 1/480000 with distilled water was carried out to make a series of successive dilutions giving a mortality ranging between 1% and 99% of Aedes aegypti larvae in contact with chlorpyrifos-ethyl. The LC50 of the chlorpyrifos-ethyl on Aedes aegypti larvae was 4.4 µg/L.

As far as water samples are concerned, a volume of 5 ml was added to the contents of a polystyrene goblet containing 5 ml of water with 20 first stage larvae of Aedes aegypti (three repetitions for each sample). The counting of mortality was made 24 hours after application.

2.2.2 Nitrates, Nitrites and Phosphates (NO3−)

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As indicated, we suppose that fertilizers used by farmers could play a role in *Aedes aegypti* larvae mortality, thus influencing the reliability of the bio-indicator. Accordingly, soil and water samples were controlled for these molecules.

The nitrates contents in soils and water samples were measured by the cadmium reduction method (Centre d’Expertise en Analyse Environnementale du Québec, 2004). Cadmium reduces nitrate to nitrite. The nitrite ion reacts with sulphanilic acid to form a salt of intermediate diazonium. This salt reacts with the gentisic acid to form complex colored amber. The measure is obtained to 400 nm.

### 2.2.3 Nitrites (NO$_2^-$)

The nitrites contents in soils and water samples were measured by diazotization (Centre d’Expertise en Analyse Environnementale du Québec, 2004). The nitrite present reacts with the sulphanilic acid to form an intermediate salt of diazonium. This salt combines with the chromotropic acid to produce a pink complex of color whose intensity is directly proportional to the nitrite concentration in the solution. The measure is obtained to 507 nm.

### 2.2.4 Phosphates (PO$_4^{3-}$)

The phosphates contents in soils were measured by the method of reduction per ascorbic acid after mineralization (Centre d’Expertise en Analyse Environnementale du Québec, 2007). Orthophosphate reacts with molybdate in acid medium to produce a mixed complex phosphate-molybdate. Then, ascorbic acid reduced the complex, causing a strong blue coloring due to molybdenum. The measure is obtained to 880 nm.

### 2.3 Statistical analysis

The SPSS 12.0 software was used to carry out the statistical analysis. A multiple linear regression was used to identify the parameters determining the mortality of the first stage larvae of *Aedes aegypti* in contact with the soils samples taken on market-gardening sites.

### 3. RESULTS AND DISCUSSION

#### 3.1. Evolution Trend of the residual contents of pesticides in the cabbage sheets and market-gardening soils before and after treatment with deltamethrin or chlorpyrifos-ethyl

Using of *Aedes aegypti* larvae to measure trends of pesticide residual content in cabbage and market-gardening soils (field trials)

At the time T = -1h, 1 hour before the spreading treating with PLAN D-25EC (deltamethrin 25 g/L), no residue of pesticide was detected in cabbage leaves samples (Fig 1). About 0.073±(0.007) ppb of equivalent-deltamethrin in toxic residues was detected in soils samples 1 hour before treatment (Fig 2).
Figure 1: Evolution Trend of the residual concentration of pesticides in the cabbage after a treatment with chlorpyrifos-ethyl or deltamethrin.
At the times \( T=+1\text{h} \) (one hour after treatment) and \( T=+1\text{d} \) (one day after treatment), the residual concentrations of deltamethrin detected in the cabbage leaves were 45\pm(1.8) ppb (45 ng/g of leaves) whereas it was about 32.5\pm(0.3) ppb [2.5 ng/g of soils or 59\pm(7.9) µg/m\(^2\)] in the soils at \( T=+1\text{h} \). These residual concentrations of deltamethrin decreased with time in the cabbage leaves and reached 3.8\pm(0.5) ppb until time \( T=+8\text{d} \). No residue was detected beyond \( T=+8\text{d} \) (eight days after treatment).

In the soils samples, the concentration of deltamethrin decreased slightly and persisted beyond \( T=+10\text{d} \) (ten days after treatment) when it reached 0.45\pm(0.03) ng/g or 9.2\pm(0.61) µg/m\(^2\). These concentrations measured are below the recommended lower than maximum tolerable residual concentration of deltamethrin in vegetables which is about 500 ppb (0.5 mg/kg) (FAO and WHO, 2012). Thus a strict respect of recommended pesticides dosages recommended by vegetable growers should result in residual concentration of deltamethrin below the recommended threshold inferior of the maximum acceptable concentration in vegetables. The detection limit of the bio-indicator was 3.6\pm(0.43) ppb (ng/g of vegetables) of equivalent-deltamethrin for in vegetable samples and 0.09\pm(0.01) µg/L of equivalent-deltamethrin for in water samples whereas the limits of detection (LOD) of deltamethrin and quantification (LOQ) for pesticides in vegetables for with certain some chromatographic methods is 50 ppb in vegetables (Saethre, M.G. et al., 2011) were 2-10 ng/g and 6-20 ng/g respectively in a study by (Osman, K.A. et al. 2010). The detection limit of this bio-indicator in water samples was comparable to those reported for chromatographic methods in a study by López-Blanco, M.C. et al. (2002). In fact this author reported that the limits of detection for carbosan, in water with high-performance liquid chromatography-photodiode-array detection were 0.06 µg/L and 8.9 µg/L for solid-phase extraction (SPE) and solid-phase microextraction (SPME) respectively (López-Blanco, M.C. et al., 2002). Also the preharvest interval of deltamethrin is was 7 days. The bioindicator detected the presence of deltamethrin in cabbage until the eighth day after treatment with the recommended dosage. Thus *Aedes aegypti* as this bio-indicator of detection constitutes is an adequate tool for the qualitative evaluation of vegetables taken from fields treated with deltamethrin. The concentration of deltamethrin measured in soils at \( T=+1\text{d} \) (a day after treatment) was lower than that reported by Hill, B.D., Johnson, D.L. (1986) on litter in a Canadian field which was 146 µg/m\(^2\) at a day after aerial application of deltamethrin. The \( DT_{90} \) for deltamethrin was 5...
days on soils which is below that reported on litter in Canada (17 days). Difference observed could be explained by technique of pesticide application, amounts of active ingredients applied, climate, temperature and soils characteristics (Wauchope, R.D. et al., 1992).

As far as PYRIFORCE 480EC is concerned, at the time T=-1h (1 hour before treatment), no residue of pesticide was detected in cabbage samples (Fig 1). At the same time, about 0.003±0.0018 ppm of equivalent-chlorpyrifos-ethyl of toxic residues was detected in soils (Fig 3).

**Figure 3:** Evolution Trend of toxic residues concentration in soils before and after treatment with chlorpyrifos-ethyl (T=0)
From time $T=+1h$ to $T=+1d$, the residual concentrations of chlorpyrifos-ethyl detected in cabbage were about $1.16\pm(0.14)$ ppm (mg/kg of leaves) whereas they were about less than $0.5\pm(0.09)$ ppm (mg/kg of soils) in the soils. These residual concentrations of chlorpyrifos-ethyl decreased with time in cabbage leaves until the fourth day after treatment. No residue was detected beyond $T=4d$ (four days after treatment).

In the soils, the concentration of chlorpyrifos-ethyl decreased slightly and persisted beyond $T=+10d$ (ten days after treatment). The concentrations of chlorpyrifos-ethyl detected in cabbages at times $T=+1h$ and $T=+1d$ were above the recommended maximum tolerable admissible concentrations in vegetables cabbage which were is about 0.1 to 0.5 $1$ mg/kg of chlorpyrifos-ethyl according to the codexalimentarius (Sæthre, M.G. et al., 2011) (FAO and WHO, 2012). The limits of detection of the bio-indicator were $0.06\pm(0.008)$ mg/kg of vegetables and $1.48\pm(0.19)$ µg/L in water whereas it was $0.02-0.010$ mg/kg for certain chromatographic methods (Osman, K.A. et al., 2010). Concentration of chlorpyrifos-ethyl measured in marketed cabbage from market reported by Osman, K.A. et al. (2010) was 6.207 mg/Kg and above those detected with this method. The bio-indicator could not detect chlorpyrifos-ethyl concentration below 0.06 mg/kg in vegetables samples. The concentrations of organophosphate measured in vegetables in most studies were above the detection limit of this bio-indicator for chlorpyrifos-ethyl (Baig, S.A., 2009). The preharvest interval of PYRIFORCE 480EC on vegetables was 14 days whereas the bio-indicator allowed to detect the presence of chlorpyrifos-ethyl less than 5 days after treatment with recommended dosage. Thus this bio-indicator is less sensitive for the detection of the chlorpyrifos-ethyl compared with to deltamethrin, but it remains a tool able to detect or not the presence of pesticides residues in vegetables only treated with pyrethroid or organophosphate or binary insecticides (pyrethroid and organophosphates).

The concentration of chlorpyrifos-ethyl measured in soils at $T=+1d$ (a day after treatment) was $0.27\pm(0.016)$ mg/kg and was higher than those measured by Kihampa, C. et al. (2010) in tomato fields' soils which were ranged from 0.09 to 0.172 mg/kg. The limit of detection for chlorpyrifos-ethyl in soil with this bio-indicator ($0.0015\pm(0.00014)$ mg/kg) was inferior to results reported by Kihampa, C. et al. (2010). Even if this bio-indicator is able to detect amount of chlorpyrifos-ethyl in soil much lower than those reported by Kihampa, C. et al. (2010), soils samples collected one hour before treatment with deltamethrin or chlorpyrifos-ethyl appeared positive with respective toxic residual concentrations of 0.073 ppb and 0.003 ppm respectively. These results suppose that apart from pesticides residues, other factors present contained other than pesticide residues in these soils like fertilizers could play an important role in the toxic effect part in the weak mortality noted on the first stage larvae of Aedes aegypti. Although the application of organic and inorganic fertilizers in field increase population densities of mosquito larvae in farming areas (Victor, T.J., Reuben, R., 2000), however toxic effects on immature larvae have been are recorded when fertilizer concentrations go beyond certain levels (Muturi, E.J. et al., 2007). Such Toxicological effects of mineral fertilizer on Aedes sp larvae have been investigated (Fedorova, V.G., 1989a, 1989b, 1990).

### 3.2. Using larvae of Aedes aegypti for Pesticides residues analysis of in-the market-gardening-products vegetables collected before harvest

Eight vegetable species and 20 samples of eight vegetable species were collected from taken in on the market-gardening-production sites just before harvest (Tables 1 and 2). These vegetables were solanum (Solanum macrocarpon), amaranth (Amaranthus hybridus), vernonia (Vernonia amygdalina), basil (Ocimum basilicum), turnip (Brassica rapa), cabbage (Brassica oleracea), lettuce (Lactuca sativa) and the gombo leaves (Abelmoschus esculentum).

Residues of pesticides were detected in 30% of these samples collected before harvest (Table 2). Residues were detected in samples of solanum, amaranth, vernonia, turnip and in...
cabbage. Approximately 3.5–60.0% of the samples collected on the site of Houéyiho in Cotonou were positive. In Sémé-kpodji and Ouidah, the rates of positive samples were respectively 28.6% and 12.5% respectively. The residual concentrations of pesticides vary from 5.2 to 96.6 ppb of equivalent-deltamethrin and 0.08 to 5.34 ppm of equivalent-chlorpyrifos-ethyl (Table 1). The highest residual concentrations were detected in Solanum macrocarpon.

**Table 1: Concentration of pesticide residues in vegetables collected in production areas (in ng/g of equivalent deltamethrin)**

<table>
<thead>
<tr>
<th>Market-gardening sites</th>
<th>Number</th>
<th>Samples</th>
<th>Rate of positive (%)</th>
<th>Concentration (ppb of eq-deltamethrin)</th>
<th>Concentration (ppm of equivalent chlorpyrifos-ethyl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Houéyiho</td>
<td>2</td>
<td>Solanum</td>
<td>50.0</td>
<td>96.6</td>
<td>5.34</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Amaranth</td>
<td>100.0</td>
<td>5.2</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Vernonia</td>
<td>100.0</td>
<td>23.2</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Basil</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Sémé-kpodji</td>
<td>1</td>
<td>Vernonia</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Basil</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Turnip</td>
<td>100.0</td>
<td>27.5</td>
<td>3.99</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Amaranth</td>
<td>100.0</td>
<td>9.7</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Lettuce</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Solanum</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Ouidah</td>
<td>2</td>
<td>Solanum</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Gombo leaves</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Basil</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Vernonia</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Cabbage</td>
<td>50.0</td>
<td>5.5</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Nd : Not detected

This should be related to massive attack of this specie by red mites (*Tetranychus sp*) in southern Benin that involve a strong use of pesticide by farmers. These results of this study confirm those of Sæthre, M.G. *et al.* (2011) which found high amounts of omethoate in *Solanum macrocarpon* in southern Benin. The maximum tolerable admissible concentrations of pesticides residues in vegetables and fruits vary from 0.1 to 1 mg/kg or 100 to 1000 ppb (FAO and WHO, 2012) and 100 ppb and 500 ppb for chlorpyrifos-ethyl respectively in carrot and tomato respectively and 500 ppb for deltamethrin in vegetables (Sæthre, M.G. *et al.*, 2011). The detected residual concentrations should be below the maximum tolerable admissible concentrations if these vegetables had been only treated with pyrethroids, but could exceed these limits if they solanum, vernonia and turnip had been treated with organophosphates. Since generally the vegetable growers in southern Benin generally use dual—several pesticides, often formulations of pyrethroid and organophosphate to control pests (Ahouangninou, C. *et al.*, 2011), the residual contents in these species could exceed the maximum residue levels (MRLs). The detected vegetable samples detected negatively could also contain concentration of organophosphate lower than 0.06 ppm. Thus the residues concentrations measured should be slightly above the maximum tolerable amounts of residues in vegetables, but. These values Residual contents...
measured in the three species were lower than those measured in vegetables before harvest in the rural city of Tori-Bossito in southern Benin (Ahouangninou, C. et al., 2012) and in the market-gardening perimeter of Toligbé in southern Benin (T. Martin, IRD-UR016, Unpublished results). These vegetables could be found on the markets or sold to consumers who buy these products directly on the production sites because these vegetables were mature for consumption. Pesticide residue analysis in vegetables collected just before harvest could be frequently carried out using this bio-indicator to know which quality of food go to market in order to prevent health risks to consumers.

3.3. Using of *Aedes aegypti* larvae for pesticides residual contents analysis in the of vegetables sold at collected in the markets of in Cotonou

Fourteen vegetable species and Forty samples of fourteen vegetable species were collected in six markets of Cotonou and its peripheries (Tables 32).

**Table 32:** Concentration of pesticide residues in vegetables collected in markets of Cotonou (in ng/g of equivalent deltamethrin)

<table>
<thead>
<tr>
<th>Markets</th>
<th>Number</th>
<th>Samples</th>
<th>Rate of positive (%)</th>
<th>Concentration (ppb of eq-deltamethrin)</th>
<th>Concentration (ppm of eq-chlorpyrifos-ethyl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Godomey</td>
<td>1</td>
<td>Lettuce</td>
<td>100.0</td>
<td>14.6</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Carrot</td>
<td>0.0</td>
<td>Nd</td>
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<tr>
<td></td>
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<td>Tomato</td>
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<tr>
<td></td>
<td>1</td>
<td>Cabbage</td>
<td>0.0</td>
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<td>Nd</td>
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<tr>
<td></td>
<td>1</td>
<td>Amaranth</td>
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<td>Nd</td>
</tr>
<tr>
<td>Kindonou</td>
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<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
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<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Carrot</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Solanum</td>
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<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td>Haie-vive</td>
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<td>Cabbage</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
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<tr>
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<td>2</td>
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<td>2.3</td>
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<td>Carrot</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Sweet-pepper</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
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<tr>
<td></td>
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<td>Spinach</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
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<tr>
<td></td>
<td>1</td>
<td>Beet</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
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<tr>
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<td>1</td>
<td>Onion</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
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<tr>
<td>Gbégamé</td>
<td>1</td>
<td>Carrot</td>
<td>0.0</td>
<td>Nd</td>
<td>Nd</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Basil</td>
<td>100.0</td>
<td>4.4</td>
<td>0.05</td>
</tr>
</tbody>
</table>

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The fourteen vegetable species collected in markets of Cotonou were lettuce (*Lactuca sativa*), tomato (*Lycopersicon esculentum*), carrot (*Daucus carota*), cabbage (*Brassica oleracea*), amaranth (*Amaranthus hybridus*), solanum (*Solanum macrocarpon*), sweet pepper (*Capsicum annuum*), spinach (*Spinacia oleracea*), beet (*Beta vulgaris*), onion (*Allium cepa*), turnip (*Brassica rapa*), cucumber (*Cucumis sativus*), vernonia (*Vernonia amygdalina*) and basil (*Ocimum basilicum*) (Tables 3-2). Residues of pesticide were detected in 3-8-12.5 % of vegetables collected from markets of Godomey, Haie-vive, Gbégamey and St. Michel (Table 4). No residue of pesticides was detected in the samples collected on the markets of Kindonou and Ganh. The pesticide residues of pesticides were detected in lettuce, basil, tomato and sweet pepper, the other tested crops showed no residues. The residuals concentrations averages vary from 2.1 to 14.6 8.8 (8.3) ppb of equivalent-deltamethrin or 0.03 to 0.15–0.1 (0.07) ppm of equivalent-chlorpyrifos-ethyl (Table 3-2).

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Maximum residue content was recorded in lettuce (14.6 of equivalent-deltamethrin or 0.15 of equivalent-chlorpyrifos-ethyl).

These amounts of pesticide residues were lower than recommended maximum tolerable residue levels (MRLs) recommended for all in-vegetables except for lettuce collected at Godomey’s market if this lettuce contained had been treated with organophosphate even if these vegetables had been treated with organophosphates. These amounts measured residues were also below the amounts of pesticide residues measured by results of Saethre, M.G. et al. (2011) in vegetable samples in southern Benin. However, the vegetables tested negatively. The marketed vegetable detected negative could contain concentrations of organophosphate lower than 0.06 ppm or concentrations of other pesticides that were not toxic for first stage larvae of Aedes aegypti. The pesticide residual levels different between species depend mostly on the harvest time (Pose-Juan, E. et al., 2006; González-Rodriguez, R.M. et al., 2008; Osman, K.A. et al., 2010) and amounts of active ingredients applied on culture. The concentrations determined measured were in general all marketed vegetables collected were lower than those recorded by Amoah, P. et al. (2006); González-Rodriguez, R.M. et al. (2008) and Osman, K.A. et al. (2010) in marketed vegetable sampled from markets and lower than those found by Rial Otero, R. et al. (2002) in grape samples. The previous reports combined with results of this study show that continuing education and awareness raising efforts towards producers about health hazards derived from pesticides made a difference and would have to be continued for sustainable vegetable production (Probst, L. et al., 2012b). Regular pesticides residues analysis in of vegetables consumed using Aedes aegypti larvae as bio-indicator will have to be made in to complement an of the awareness campaign. Chromatographic analysis coupled with mass spectrometry could specify the molecules and identify possible residues of organophosphates or fungicides pesticides to which first stage larvae of Aedes aegypti are less sensitive. The promotion of the use of natural extracts of bio-pesticides plants in the control of pests could also limit the risk of presence of pesticides residues in vegetables sold in markets (Adekambi, S.A. et al., 2010; Lund, T. et al., 2010; Birch, A.N.E. et al., 2011).

3.4. Using larvae of Aedes aegypti for pesticide residues analysis in soils and water, taking into account of pesticides, nitrates, nitrites and phosphates in the soils and water coming from the market-gardening sites

The detection limit of this bio-indicator in water samples [0.09±0.01] µg/L of equivalent-deltamethrin or 1.48±0.19) [µg/L of equivalent-chlorpyrifos-ethyl] was comparable to those reported for chromatographic methods in a study by López-Blanco, M.C. et al. (2002). In fact, this author reported that the limits of detection for carbofuran in water with high performance liquid chromatography-photodiode-array detection were 0.06 µg/L and 8.9 µg/L for solid-phase extraction (SPE) and solid-phase microextraction (SPME) respectively. No residue of pesticides was not detected in groundwater samples taken on the market-gardening sites. These results conflicted with those of Traoré, S.K. et al. (2006) and those of Cissé, I. et al. (2003), who detected residues of pesticides in groundwater in agricultural production zones in Ivory Coast and those of Cissé, I. et al. (2003) in groundwater in vegetable production zones of Niayes in Senegal, respectively. This difference contradiction could result from the fact that producers in study sites started applying the recommended amounts of pesticides knowing its risk on environment, otherwise these water samples would contain low concentration of pesticides residues belonging to the chemical families to which first stage larvae of Aedes aegypti is less sensitive. The average concentration in nitrates in these water samples of water were 13.45±2.13 mg/L; 16.4±2.24 mg/L and 32.75±7.32 mg/L respectively at Sèmè-kpodji, Houéyiho and Ouidah respectively (Table 53). These nitrates concentrations in water were above of higher than those reported by Tanko, J.A. et al. (2012) in groundwater at Zaria in Nigeria, but below lower than the recommended maximum admissible amounts levels which were of 50 mg/L.

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The average concentrations in nitrites were higher at Houéyiho [0.76±(0.10) mg/L] compared to Sémé-kpodji [0.006±(0.001) mg/L] and Ouidah [0.003±(0.001) mg/L], whereas the concentration of toxic pesticide residues in soils were respectively of 1.298±(0.24) ppb; 1.475±(0.31) ppb and 0.33±(0.09) ppb of equivalent-deltamethrin at Houéyiho, Sémé-kpodji and Ouidah respectively.

Table 53: Concentration in pesticides, nitrates, nitrites and phosphates samples of water and soils from market-gardening areas

<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration (ppm d'Eq-deltamethrin)</th>
<th>Concentration in Nitrates (mg/L)</th>
<th>Concentration in Nitrites (mg/L)</th>
<th>Concentration in Phosphates (mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundwater from sites</td>
<td>(ng/g d'Eq-deltamethrin)</td>
<td>(mg/L)</td>
<td>(mg/L)</td>
<td></td>
</tr>
<tr>
<td>Houéyiho</td>
<td>Nd</td>
<td>16.40 [2.24]</td>
<td>0.76 [0.10]</td>
<td></td>
</tr>
<tr>
<td>Sémé-kpodji</td>
<td>Nd</td>
<td>13.45 [2.13]</td>
<td>0.006 [0.001]</td>
<td></td>
</tr>
<tr>
<td>Ouidah</td>
<td>Nd</td>
<td>32.75 [7.32]</td>
<td>0.003 [0.001]</td>
<td></td>
</tr>
<tr>
<td>Soils from sites</td>
<td>(ng/g d'Eq-deltamethrin)</td>
<td>(mg/Kg)</td>
<td>(mg/Kg)</td>
<td>(mg/Kg)</td>
</tr>
<tr>
<td>Houéyiho</td>
<td>1.298 [0.24]</td>
<td>544.31 [158.40]</td>
<td>3.48 [1.15]</td>
<td>2270.00 [612.81]</td>
</tr>
<tr>
<td>Sémé-kpodji</td>
<td>1.475 [0.31]</td>
<td>1118.44 [246.24]</td>
<td>2.89 [1.04]</td>
<td>772.50 [188.15]</td>
</tr>
<tr>
<td>Ouidah</td>
<td>0.33 [0.09]</td>
<td>1071.47 [226.13]</td>
<td>4.97 [1.32]</td>
<td>283.33 [103.34]</td>
</tr>
</tbody>
</table>

The concentration of nitrates in soils were lower at Houéyiho [544.31±(158.40) mg/kg of soils or 108.86±(31.70) Kg/ha] compared to those of Sémé-kpodji and Ouidah (p<0.05). These values were above higher than those reported by Assogba-Komlan, F. et al. (2007) in the soils of market gardening in the valley of Ouémé and at Cotonou in southern Benin. These results on nitrates in soils suggest supposed a risk of infiltration of nitrates in subterranean water. As far as nitrites are concerned, no significant difference at 5% was observed. The mean values were respectively of 3.48±(1.15) mg/kg; 2.89±(1.04) mg/kg and 4.97±(1.32) mg/kg at Houéyiho, Sémé-kpodji and Ouidah respectively. Higher phosphate contents in soils were observed at Houéyiho [2270±(612.81) mg/kg]. These values were significantly higher than those of Sémé-kpodji and Ouidah (p<0.05).

To determine the cause of mortality of larvae of *Aedes aegypti* in contact with soils from vegetable production sites, a multiple linear regression model was estimated. The estimated results of the analysis model of the first stage larvae mortality determinants in contact with the extracts of soils showed that the model estimate was overall significant at the 10% level, but was not at 5% (Table 64).

Table 64: Determinants of the mortality of the larvae of *Aedes aegypti* in contact with market-gardening soils

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mortality of larvae L1 of <em>Aedes aegypti</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficients</td>
</tr>
<tr>
<td>Nitrates</td>
<td>0.053**</td>
</tr>
<tr>
<td>Nitrites</td>
<td>-7.633</td>
</tr>
<tr>
<td>Phosphates</td>
<td>0.015**</td>
</tr>
<tr>
<td>Number of days after pesticide treatment</td>
<td>-0. 042*</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.846</td>
</tr>
<tr>
<td>F</td>
<td>4.42*</td>
</tr>
</tbody>
</table>

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The overall concentration of nitrates, nitrites and phosphates in soils and number of days after pesticides treatment explains 68.8% of the variation of the mortality rate observed with first stage larvae of *Aedes aegypti*. The mortality recorded with larvae in contact with the extracts of soils was not only due to the presence of pesticides in soils, but also to the concentrations of nitrates and phosphates in these samples and thus confirm results of Fedorova, V.G. (1989a, 1989b) and Muturi, E.J. *et al.* (2007). The bioindicator used is thus not limited to the detection of pesticides residues in soils of market-gardening areas, but allows the detection in these soils, of the presence of toxic elements for *Aedes aegypti* larvae. Using *Aedes aegypti* larvae as bio-indicator is inappropriate to measure pesticide residues on farms where fertilizers are regularly used. However it could be used to measure pesticide residues on lands put in fallow.

4. CONCLUSION

Pesticides residues were found in small quantity in vegetables produced and sold in Benin and also in vegetables collected just before harvest on the fields. No residue of pesticides was detected in the samples of groundwater collected on production sites. The method used makes it possible to detect insecticide residues in vegetables, 4 to 8 days after insecticidal treatment chlorpyrifos-ethyl and deltamethrin application at dosages recommended for crop protection on culture. But this method proves inappropriate to measure pesticides residues in market-gardening soils, but to suspect presence of pesticides residues because some of these soils already contain some amounts of nitrite, nitrate and phosphate, coming from the decomposition of fertilizers that are poisonous for the first stage larvae of *Aedes aegypti* beyond certain level.

Regarding the actual contamination, pesticide residues were found in small quantity in vegetables produced and sold in Benin and also in vegetables collected just before harvest on the fields. Pesticide residues were not detected in the samples of groundwater collected on production sites.

We conclude that this biological method of evaluating assessment of the contamination risk of vegetables by pesticides detected pyrethrinoid and organophosphate but proved to be more sensitive to pyrethrinoid, using larvae of *Aedes aegypti* larvae to assess pesticide contamination status is inexpensive and easy-to-use. It could be used for a monitoring program before running a more thorough chemical analysis which would then be used for identifying to identify and quantifying the pesticides molecules present in samples. We recommend Efurther researches on the sensibility of *Aedes aegypti* larvae to others families of pesticides used in vegetable growing in Benin are need to know the range of pesticides active ingredients this bio-indicator can detect. This would enable researchers to understand the range of active ingredients of pesticides the bio-indicator can detect.

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COMPETING INTERESTS

No competing interests exist.

REFERENCES


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DEFINITIONS, ACRONYMS, ABBREVIATIONS

- ng/g : nanogram per gram
- mg/Kg : milligram per kilogram
- µg/L : micogram per liter
- Eq-deltamethrin : Equivalent-deltamethrin
- Eq-chlorpyrifos-ethyl : Equivalent- chlorpyrifos-ethyl
- Nd : Not detected
- ppm : part per million
- ppb : part per billion