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Using *Aedes aegypti* larvae to Assess Pesticide Contamination of Soil, Groundwater and Vegetables

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Authors' contributions

This work was carried out in collaboration between all authors. Author CA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors TM, FA, SS, VC and BF managed the analyses of the study. Authors CA, TM, VC and LD managed the literature searches. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

In Benin, the use of synthetic pesticides in vegetable production poses a risk to the environment and human health. Vegetables, water and soil quality assessment is very important for monitoring and mitigation of these risks. The evaluation of pesticide contamination of vegetables and agricultural environment is often made using expensive

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methods. It is crucial 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 soil, groundwater and vegetables. Vegetables just before harvest, groundwater and soils samples from three production sites and vegetables samples from markets were collected from March to August 2011. Ethanol extracts of these samples were tested on first stage larvae of *Aedes aegypti*. The method made it possible to detect residues of chlorpyrifos-ethyl and deltamethrin in cabbage until 4 and 8 days respectively after treatment with the recommended doses for crop protection. It proved inappropriate to measure pesticides residues in market-gardening soils, since these soils 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*. Overall, the results revealed the presence of small amounts of pesticides residues in 12.5% of the vegetables collected from markets. Pesticides residues were also detected in 30.0% of vegetables collected just before harvest. Residues of pesticide were not detected in groundwater samples collected from vegetable growing areas. First stage larvae of *Aedes aegypti* could be used as a bio-indicator to characterize and monitor risk of pesticide contamination of vegetables in southern Benin. 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.

Keywords: Pesticides; vegetable contamination; environment; bio-indicator; Southern-Benin.

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; SD: Standard-Deviation.

1. INTRODUCTION

The production of vegetables is an important part of urban and peri-urban agriculture. It contributes to 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 [1,2] The production of vegetables reduces the level of unemployment and contributes to the creation of more than 600000 jobs in Benin [3]. The production is ensured by small-scale producers in or on the peripheries of the big cities generally occupying swamps or marshes areas for vegetable growing [4]. Although this activity presents a significant economic interest, it also has harmful effects on the environment [5,6] and on human health [7,8]. Pesticides intended for use on cotton fields are often used for vegetable crops protection [9,10]. Similarly producers do not always respect the quantities guidelines and the preharvest intervals recommended [11,12]. [13] 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. Vegetables, water and soil quality assessment is important for monitoring and mitigation of pesticide risks. To assess pesticide contamination in vegetables, foods, groundwater and soils, conventional techniques like chromatography with mass spectrometry are frequently used [14,15,16,17,18,19,20]. These conventional techniques are costly. It is a challenge for research in developing countries to develop less expensive tools for pesticide risks monitoring. Bio-indicators are living thing (animals, plants and microorganisms) used to monitor environmental health. Environmental health assessment using bio-indicators or ecotoxicological approach were reported in literature [21,22,23,24]. 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.

2. MATERIALS 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 for crop protection. No manure was added during the study period to the sampled plots.

From March to August 2011, 20 vegetable samples were collected just before harvest on the market-gardening sites of Houéyiho, Sèmè-kpodji, Ouidah. Vegetable farms selected were involved in the project “Ecosystemic Approach for Sustainable Vegetable Production Project” led by Cotonou’s Applied Biomedical Science Institute and Hortsys Unit of International Cooperation Center in Agronomic Research for Development. 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, University of Abomey-Calavi, Benin, Unpublished results). Moreover, 40 vegetable samples were collected in six markets of Cotonou and its neighborhood. These markets included in the study and sellers were selected with random method. Samples were packed and sent to laboratory of Mivegec associated to the Entomological Research Center of Cotonou for toxicological analysis. 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 Cotonou in order to determine their concentrations in nitrates, nitrites and phosphates. Indeed, we suppose that fertilizers used by farmers could play a 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, 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 done using mosquito larvae as biological indicator. The method of pesticide residues detection using a biological indicator (first stage larvae of *Aedes aegypti*) was first described by [25] to quantify pyrethroids on treated mosquito nets and used by [26] to detect pesticide 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 response observed on *Aedes aegypti* larvae to references molecules which will be called “equivalent-deltamethrin” and “equivalent-chlorpyrifos-ethyl”. The insecticide deltamethrin was used because of its strong toxicity (LC_{50} of 0.4 µg/L) on *Aedes aegypti* larvae S-Be from north Benin [26], but also because it belongs to the family of pyrethroid frequently used in vegetable crop 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 cm^3 of soils were weighed and put in a tube in which 10 ml and 100 ml of ethanol were added respectively in order to extract the pesticides residues. 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 reported by [27]. 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 series of ten successive dilutions were made to have a mortality ranging between 1% and 99%. The residual contents of pesticides present in vegetable were evaluated in nanogram per gram of equivalent-deltamethrin. 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 series of successive dilutions giving a mortality ranging between 1% and 99% of *Aedes aegypti* larvae in contact with chlorpyrifos-ethyl. The LC_{50} of the chlorpyrifos-ethyl on *Aedes aegypti* 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

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 nitrate content in soil and water samples was measured by the cadmium reduction method [28]. 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.

The nitrite content in soil and water samples was measured by diazotization [28]. 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.

The phosphate content in soil samples was measured by the method of reduction per ascorbic acid after mineralization [29]. 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 software SPSS 12.0 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 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 treating with PLAN D-25EC (deltamethrin 25 g/L), no residue of pesticide was detected in cabbage leave samples (Fig. 1). About 0.073(0.007) ppb of equivalent-deltamethrin in toxic residues was detected in soil samples 1 hour before treatment (Fig 2).

At the times T=+1h (one hour after treatment) and T=+1d (one day after treatment), the residual concentrations of deltamethrin detected in the cabbage leaves were 45(1.8) ppb (45 ng/g of leaves) whereas it was 2.5(0.3) ppb [2.5 ng/g of soils or 59(7.9) µg/m²] in soils at T=+1h. These residual concentration of deltamethrin decreased with time in the cabbage leaves and reached 3.8(0.5) ppb at time T=+8d. No residue was detected beyond T=+8d (eight days after treatment).

In the soils samples, the concentration of deltamethrin decreased slightly and persisted beyond T=+10d (ten days after treatment) when it reached 0.45(0.03) ng/g or 9.2(0.61) µg/m². Concentrations measured in cabbage were lower than maximum admissible concentration of deltamethrin in vegetables which is 500 ppb (0.5 mg/kg) [30]. Thus a strict respect of recommended pesticides dosages by vegetable growers should result in residual concentration of deltamethrin below the recommended threshold. The detection limit of the bio-indicator was 3.6(0.43) ppb (ng/g of vegetables) of equivalent-deltamethrin in vegetable samples and 0.09(0.01) µg/L of equivalent-deltamethrin in water samples that was comparable to the limits of detection (LOD) and quantification (LOQ) for pesticides in vegetables with some chromatographic methods: 2-10 ng/g and 6-20 ng/g respectively in a study by [19]. The bioindicator detected the presence of deltamethrin in cabbage until the eighth day after treatment with the recommended dosage. Thus *Aedes aegypti* as bio-indicator 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=+1d (a day after treatment) was lower than that reported by [31] on litter in a Canadian field which was 146 µg/m² at a day after aerial application of deltamethrin. The DT_{50} 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 [32].

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 residue was detected in soils (Fig 3).

From time T=+1h to T=+1d, the residual concentrations of chlorpyrifos-ethyl detected in cabbage were 1.16(0.14) ppm (mg/kg of leaves) whereas they were less than 0.5(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).

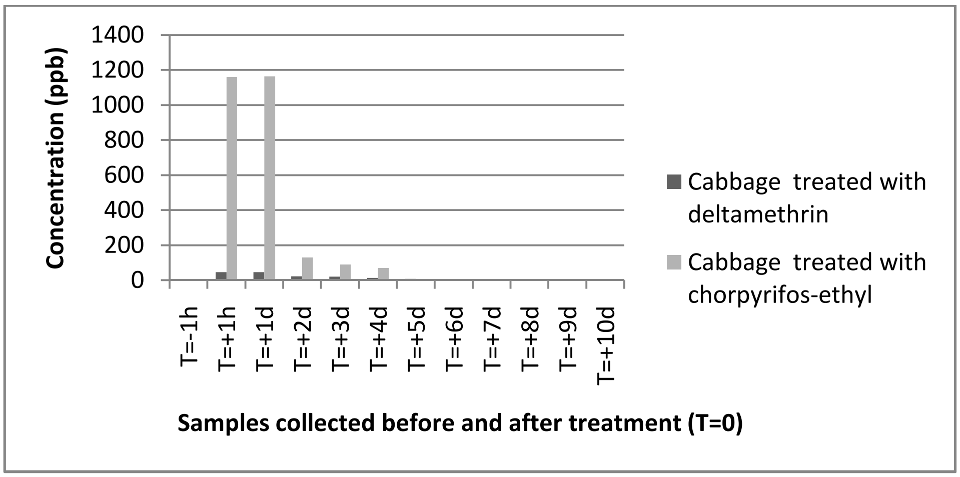

Fig. 1. Trend of the residual concentration of pesticides in cabbage after a treatment with chlorpyrifos-ethyl or deltamethrin

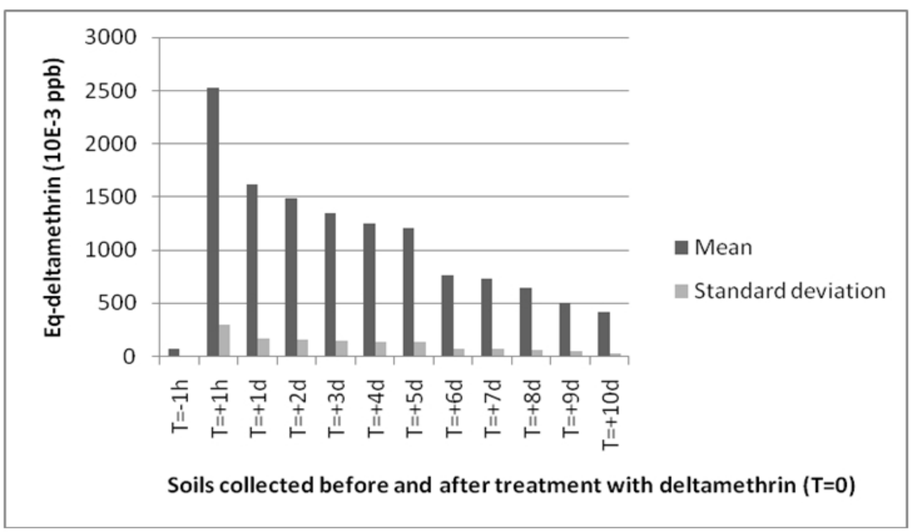

Fig. 2 . Trend of residual concentration in soil before and after treatment with deltamethrin

In the soil samples, 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 admissible concentration in cabbage which is 1 mg/kg of chlorpyrifos-ethyl according to the codexalimentarius [30]. The limits of detection of the bio-indicator were 0.06(0.008) mg/kg of vegetables and 1.48(0.19) µg/L in water that were above those of certain chromatographic methods: 0.002-0.010 mg/kg [19]. Concentration of chlorpyrifos-ethyl measured in cabbage from market reported by [19] 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, but the concentrations of organophosphate measured in vegetables in most studies were above the detection limit of this bio-indicator for chlorpyrifos-ethyl [19,33]. The preharvest interval of PYRIFORCE 480EC on vegetables is 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 to deltamethrin, but it remains a tool able to detect the presence of pesticides residues in vegetables treated with pyrethroid or organophosphate. Soil samples collected one hour before treatment with deltamethrin or chlorpyrifos-ethyl appeared positive with residual concentrations of 0.073 ppb and 0.003 ppm respectively. These results suggest factors other than pesticides residues play a role in the toxic effect on the first stage larvae of *Aedes aegypti*. Although the application of organic and inorganic fertilizers increase population densities of mosquito larvae in farming areas [34,35,36], toxic effects on immature larvae have been recorded when fertilizer concentrations go beyond certain levels [37] Such Toxic effects of mineral fertilizer on *Aedes sp* larvae have been investigated [38,39,40].

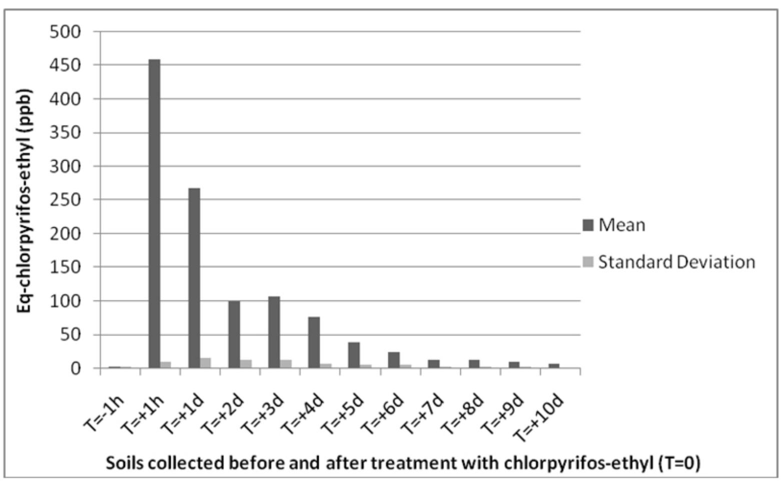

Fig. 3. Trend of residual concentration in soil before and after treatment with chlorpyrifos-ethyl

3.2 Using Larvae of *Aedes aegypti* for Pesticides Residues Analysis of Vegetables Collected before Harvest

Twenty samples of eight vegetable species were collected from production sites just before harvest (Table 1). 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. Residues were detected in samples of solanum, amaranth, vernonia, turnip and in cabbage. Approximately 60.0% of the samples collected on the site of Houéyiho in Cotonou were positive. In Sèmè-kpodji and Ouidah, the rates of positive samples were 28.6% and 12.5% respectively. The residual concentrations of pesticides vary from 5.2 to 96.6 ppb of equivalent-deltamethrin or 0.08 to 5.34 ppm of equivalent-chlorpyrifos-ethyl (Table 1). The highest residual concentration was detected in *Solanum macrocarpon.*

Table 1. Concentration of pesticide residues in vegetables collected in production areas

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

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. The results of this study confirm those of [41] which found high amounts of omethoate in *Solanum macrocarpon* in southern Benin. The maximum admissible concentration of pesticides residues in vegetables and fruits vary from 100 ppb and 500 ppb for chlorpyrifos-ethyl in carrot and tomato respectively and 500 ppb for deltamethrin in vegetables [30]. The detected residual concentrations in solanum, vernonia and turnip should be below the maximum admissible concentrations if these vegetables had been only treated with pyrethroids, but could exceed these limits if they had been treated with organophosphates. Since the vegetable growers in southern Benin generally use several pesticides, often formulations of pyrethroid and organophosphate to control pests [10], the residual contents in these species could exceed the maximum residue levels (MRLs). The samples detected negatively could also contain concentration of organophosphate lower than 0.06 ppm. 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 [26] 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 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 Pesticide Residue Analysis of Vegetables Sold at Markets in Cotonou

Forty samples of fourteen vegetable species were collected in six markets of Cotonou and its peripheries (Table2).

Table 2. Concentration of pesticide residues in vegetables collected in markets of Cotonou

Samples	Number	Rate of positive (%)	Concentration (ppb of eq-deltamethrin)	Concentration (ppm of eq-chlorpyrifos-ethyl)
Amaranth	1	0.0	Nd	Nd
Basil	1	100.0	4.4	0.05
Beet	1	0.0	Nd	Nd
Cabbage	6	0.0	Nd	Nd
Carrot	6	0.0	Nd	Nd
Cucumber	1	0.0	Nd	Nd
Lettuce	7	28.6	8.8 (8.3)	0.1 (0.07)
Onion	2	0.0	Nd	Nd
Solanum	4	0.0	Nd	Nd
Spinach	1	0.0	Nd	Nd
Sweet pepper	3	33.3	2.1	0.03
Tomato	4	25.0	4.4	0.05
Turnip	1	0.0	Nd	Nd
Vernonia	2	0.0	Nd	Nd

Nd: Not detected

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*) (Table 2). Residues of pesticide were detected in 12.5 % of vegetables collected from markets. The pesticides residues were detected in lettuce, basil, tomato and sweet pepper, the other tested crops showed no residues. Residuals concentrations averages vary from 2.1 to 8.8 (8.3) ppb of equivalent-deltamethrin or 0.03 to 0.1 (0.07) ppm of equivalent-chlorpyrifos-ethyl (Table 2). 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 maximum residue levels (MRLs) for all vegetables except for lettuce collected at Godomey market if this lettuce had been treated with organophosphate. The measured residues were also below the results of [41]. However, the vegetables tested negatively could contain concentrations of organophosphate lower than 0.06 ppm or concentrations of other pesticides that are not toxic to first stage larvae of *Aedes aegypti*. The concentrations measured were in general lower than those reported by [17,19] in vegetable sampled from markets and by [15] in grape samples. The results of this study show that continuing education and awareness raising efforts towards producers about health hazards derived from pesticide would have to be continued for sustainable vegetable production [42]. Regular pesticides residues analysis of vegetables from market using *Aedes aegypti* larvae as bio-indicator will have to be made to complement an awareness campaign. Chromatographic analysis coupled with mass spectrometry could specify the molecules and identify possible residues of pesticides to which first stage larvae of *Aedes aegypti* are less sensitive. The promotion of the use of natural extracts of bio-pesticides for control of pests could also limit the risk of presence of pesticides residues in vegetables sold in markets [13,43].

3.4 Using Larvae of *Aedes aegypti* for Pesticide Residues Analysis in Soils and Water, Taking into Account Nitrates, Nitrites and Phosphates

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 [14]. 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. Pesticides were not detected in groundwater samples taken on the market-gardening sites. These results conflict with those of [44,45], who detected residues of pesticides in groundwater in agricultural production zones in Ivory Coast and Niayes in Senegal, respectively. This 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 could contain low concentration of pesticides residues belonging to the chemical families to which first stage larvae of *Aedes aegypti* is less sensitive. The results on nitrates in soils suggest a risk of infiltration of nitrates in subterranean water. The average concentration in nitrates in water samples were 13.45(2.13) mg/L; 16.40(2.24) mg/L and 32.75(7.32) mg/L at Sèmè-kpodji, Houéyiho and Ouidah respectively (Table 3). These nitrates concentrations in water were higher than those reported by [46] in groundwater at Zaria in Nigeria, but lower than the maximum admissible levels which are 50 mg/L [30]. The concentration of pesticide residues in soils were 1.298(0.24) ppb; 1.475(0.31) ppb and 0.33(0.09) ppb of equivalent-deltamethrin at Houéyiho, Sèmè-kpodji and Ouidah respectively.

The overall concentration of nitrates, nitrites and phosphates in soils and number of days after pesticide 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 [37,38,39]. Using *Aedes aegypti* larvae as bio-indicator is inappropriate to measure pesticide residues on farms soils where fertilizers are regularly used. However it could be used to measure pesticide residues on lands put in fallow.

Table 3. Concentration in pesticides, nitrates, nitrites and phosphates samples of water and soils from market-gardening areas

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

SD: Standard-Deviation

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 model estimate was overall significant at the 10% level (Table 4).

Table 4. Determinants of the mortality of the larvae of *Aedes aegypti* in contact with market-gardening soils

Parameter	Mortality of larvae L1 of *Aedes aegypti*	
	Coefficients	**Standard-Error**
Nitrates	0.053**	0.02
Nitrites	-7.633	4.203
Phosphates	0.015**	0.005
Number of days after pesticide treatment	-0. 042*	0.03
Intercept	1.846	31.086
F	4.42*	
R^2-ajusted	0.688	

**, ** indicate significative at 10%, 5%*

4. CONCLUSION

The method used makes it possible to detect insecticide residues in vegetables, 4 to 8 days after chlorpyrifos-ethyl and deltamethrin application at dosages recommended for crop protection. It proves inappropriate to measure pesticides residues in market-gardening soils due to the presence of pesticides residues, nitrite, nitrate and phosphate, coming from the decomposition of fertilizers that may affect the outcomes, i.e mortality of *Aedes aegypti* larvae.

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

We conclude that using larvae of *Aedes aegypti* to assess pesticide contamination status is inexpensive and easy. It could be used for a monitoring program before running a more thorough chemical analysis to identify and quantify the pesticide molecules present in samples. We recommend future research on the sensibility of *Aedes aegypti* larvae to other families of pesticides used in vegetable growing in Benin. This would enable researchers to understand the range of active ingredients of pesticides the bio-indicator can detect.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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