Gerold Rahmann, Victor Olowe, Timothy Olabiyi, Khalid Azim, Olugbenga AdeOluwa (Eds.) (2018) Scientific Track Proceedings of the 4TH African Organic Conference. "*Ecological and Organic Agriculture Strategies for Viable Continental and National Development in the Context of the African Union's Agenda 2063*". November 5-8, 2018. Saly Portudal, Senegal

Determination of Pesticide Residues in Organic and Conventional Exotic Vegetables

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Keywords:
Pesticide residues, Vegetables, Cypermethrin, Lambda-cyhalothrin

Abstract

There have been concerns over indiscriminate use of pesticides by farmers to grow vegetables especially for local markets since there are no guidelines on Maximum residue levels. This study was done to determine the concentration of cypermethrin and lambda-cyhalothrin pesticide residues in Collard (Brassica oleracea var. acephala) Tomatoes (Solanum lycopersicum) and swiss chard (Beta vulgaris subsp. cicla). The samples included both organic and conventional vegetables that use chemical pesticides. Experimental study design was used which involved laboratory analysis of the samples. Sample extraction was done using AOAC official method 2007.01 known as Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) method. The method involves use of Acetonitrile, sodium chloride and anhydrous magnesium sulphate for extraction. Clean-up was done using dispersive-solid phase extraction method using Primary-Secondary Amine and anhydrous magnesium sulphate. Residues analysis was done using Reverse-phase High Performance Liquid chromatography. Peak areas of the curves were calculated using Motic Images plus 2.0 and data analysis was done using SPSS 22. Recovery rates of pesticide ranged from 87.78% to 97.93% for cypermethrin and 90.65% to 95.72% for lambda-cyhalothrin. The results indicated that organic vegetable samples had pesticide residues below detectable levels while conventional vegetable samples had levels ranging from 2.495mg/kg to 0.238mg/kg for cypermethrin and 0.352mg/kg to 0.119mg/kg for lambda-cyhalothrin. The residues of both pesticides were above the recommended, this is likely to cause negative health effects such as uncoordination, whole-body tremors and seizures. This indicates that conventional vegetable consumers are exposed to pesticide residues. Farmers should strictly adhere to good agricultural practice to reduce pesticide residues.

Introduction

Pests and plant diseases have been a problem to vegetable farmers and a threat to food security globally as they destroy crops and reduce yields hence financial losses. Pests are organisms that feed on plants as a source of food (Ata *et al*., 2013). Pests can be carriers of plant pathogens which also exist in soil. Farmers use pesticides to control the pests and plant diseases. Pesticides have been in use since early years by Sumerians where they used compounds of sulphur to control mites and insects 4500 years ago. They also used mercury, lead, zinc and arsenical compounds to grow vegetables and fruits (Unsworth, 2010). Before the invention of chemical industries, most pesticides were derivatives of plants and animals (Fishel, 2013).

Chemical pesticides are widely used globally as they are perceived to be more effective and relatively cheap. Kenya being a country that widely relies on agriculture, there is widespread use of chemical pesticides to control plant diseases and pests. This is in effort to improve yield and produce blemish-free products. Emergence of new diseases and pests has led to the need of better pesticides to counter this problem. Farmers are encouraged to embrace new methods of controlling plant diseases and pests such as integrated pest management to reduce reliance on chemical pesticides. Despite this effort, use of chemical pesticides is still wide spread in the country.

Most chemical pesticides cause negative health effects to human beings especially if exposed to high doses. They can affect the respiratory system, nervous system and interfere with the function of cell membrane of target and non-target organisms (Corbett, 1974). For this reason, it is important to prevent excessive exposure of humans to pesticides. Occupational exposure to these pesticides can be controlled by educating the handlers on use of protective equipment during usage. Dietary exposure to pesticides through residues can also be controlled by ensuring that farmers adhere to good agricultural practices, practicing organic agriculture and complying with the recommended maximum residue levels (MRLs).

The objective for this study was to determine the concentration of pesticide residues in locally consumed vegetables. The hypothesis was that pesticide residues in locally consumed vegetables were above the recommended Maximum residue Levels.

Materials and Methods

Study Areas

Nakuru town and parts of Nairobi were the study areas included in the study. Three open air markets in Nakuru town were selected, they included, Soko-mjinga, Ponda-mali and Main Municipal Markets for conventional vegetables. Kalimoni Greens, Carre four supermarket and Karen Organic Market located in Nairobi for organic vegetables also included in the study. The two study areas were chosen since they had similarities in pesticide use.

Sampling of vegetables for analysis

The vegetables included in the study were Collard greens (*Brassica oleracea var. acephala*), Tomatoes (*Solanum lycopersicum*) and Swiss chard (*Beta vulgaris subsp. cicla*). The samples included both conventional and organically grown vegetables. The vegetables were randomly sampled and were bought in triplicates. They were packaged in polythene bags, labelled and stored at temperatures below $5^{\circ}C$ for transportation to the laboratory.

Extraction of Pesticide residues from vegetables

Quick, Easy, Cheap, Effective, Rugged and Safe Method also known as the QuEChERS was used in the analysis of pesticide residues (Anastassiades *et al.,* 2003). This method is also registered as AOAC 2007.01 official method for pesticide residue analysis. Triplicates of vegetable samples were chopped and shredded in a blender to obtain a homogeneous composite sample. 10g of the homogenized sample was weighed into 50mL Teflon centrifuge tube. 10mL of MeCN was added using a dispenser (pipet), and the sample was vigorously shaken for 1min using a Vortex mixer at maximum speed. 4 g anhydrous $MgSO_4$ and 2g NaCl was added and mixed immediately on a Vortex mixer for 1 min. This was done immediately to prevent formation of $MgSO_4$ conglomerates. 40 mL Internal standard solution was added, mixed on a vortex mixer for another 30 s, and extract was centrifuged for approximately 5 min at 5000 rpm. 1 mL aliquot of upper MeCN layer was transfered into 1.5 mL microcentrifuge test tube containing 25 mg PSA sorbent and 150 mg anhydrous $MgSO_4$ and capped. The mixture was then shaken with Vortex mixer for 30 s. Extracts were centrifuged for 1 min at 6000 rpm to separate solids from solution, and 0.5 mL of extract was transferred to HPLC analysis.

High performance liquid chromatography machine Waters 600 Controller model was used for analysis. The machine was connected to a detector Waters 484 Tunable Absorbance Detector model.

Acetonitrile and water were used as mobile phase in a ratio of 80:20 v/v respectively. The column (LiChrospher® 100 Rp-18, 5µm) was at room temperature while the flow rate was 1ml/min. The wavelength of the detector was set at 205nm with a sensitivity of 0.5. 20µL of extract was injected into the HPLC machine for analysis using a micro-syringe.

Data Analysis

Peak areas were calculated using Motic Images plus 2.0. The formula used was; Peak area=concentration of pesticide residues. The data was then transferred to Microsoft Excel for more analysis.

Results

Recovery Assays

Fresh vegetable samples (Collard greens, tomatoes and swiss chard) were gotten from a home vegetable garden that has never been sprayed with pesticides. They were used in testing the recovery rates of the pesticides using the QuEChERS method. The samples were first analysed to ascertain that they really had no pesticide residues since the farmers do not use chemical pesticides. The vegetables were spiked with known concentration of pesticide standards. Extraction of pesticide was done using the procedure and analysed to determine the recovery rate. This was done in triplicates for the three types of vegetables. The results of the recovery percentages from the vegetables were as indicated in Table 1.

Table 1. **Mean Recovery rates of pesticides**

Vegetables	Cypermethrin (% Mean±SD)	Lambda-Cyhalothrin (% Mean±SD)
Collard greens	95.7±0.01	91.97±0.019
Tomatoes	87.78±0.014	90.65±0.049
Swiss chard	97.93±0.006	95.72±0.023

Concentration of lambda-cyhalothrin and Cypermethrin in vegetables

Table 2. **Concentration of Cypermethrin and Lambda-Cyhalothrin residues**

Markets	Vegetables	Cypermethrin (Mean±SD)mg/kg	Lambda-cyhalothrin (Mean±SD)mg/kg
Soko-mjinga	Collard greens	1.397±0.478	0.262±0.108
Ponda-mali	Collard greens	0.982±0.265	0.341±0.164
Main Municipal Market	Collard greens	0.238±0.132	0.219±0.427
Kalimoni Greens	Collard greens	B.D.L	B.D.L
Carre Four	Collard greens	B.D.L	B.D.L
Karen Organics	Collard greens	B.D.L	B.D.L
Soko-mjinga	Swiss chard	2.458±0.298	0.352±0.193
Ponda-mali	Swiss chard	2.495±0.109	0.24±0.045
Main Municipal Market	Swiss chard	1.462±0.239	0.28±0.365
Kalimoni Greens	Swiss chard	B.D.L	B.D.L
Carre Four	Swiss chard	B.D.L	B.D.L
Karen Organics	Swiss chard	B.D.L	B.D.L
Soko-mjinga	Tomatoes	0.232±0.085	0.081±0.037
Ponda-mali	Tomatoes	0.296±0.076	0.046±0.049
Main Municipal Market	Tomatoes	0.401±0.052	0.119±0.052
Kalimoni Greens	Tomatoes	B.D.L	B.D.L
Carre Four	Tomatoes	B.D.L	B.D.L
Karen Organics	Tomatoes	B.D.L	B.D.L

Generally, the results indicated that the concentration of pesticide residues in all organically grown vegetable samples were below detectable levels while conventional vegetable samples had pesticide residues as shown in table 2. The concentration of cypermethrin residues was higher in all conventional vegetable samples than lambda-cyhalothrin. The concentration of cypermethrin in conventional composite vegetable samples ranged between 0.232 and 2.495 mg/kg with a mean of 1.107±0.912 mg/kg. On the other hand, the concentration of lambda-cyhalothrin ranged between 0.046 and 0.352mg/kg with a mean of 0.216±0.11 mg/kg.

It was also noted that swiss chard had the highest concentration of pesticide residues among all the vegetables. The concentration of pesticide residues in swiss chard ranged between 1.462 and 2.495 mg/kg for cypermethrin and between 0.352 and 0.24 mg/kg for lambda-cyhalothrin. On the other hand, tomatoes had the lowest residues of both pesticides which ranged between 0.232 and 0.401 mg/kg for cypermethrin and between 0.046 and 0.119 mg/kg for lambda-cyhalothrin.

Discussion

The concentration of pesticide residues in all organic vegetable samples were below detectable levels. This hence confirms that farmers practicing organic agriculture adhere to the principles of organic agriculture which discourages use of chemical pesticides. Hence organic produce are safer since during analysis, there were no peaks produced to indicate that there were other detectable pesticides.

The concentration of Lambda-cyhalothrin in conventional vegetable samples were lower than Cypermethrin. This is because farmers may be spraying more Cypermethrin oftenly than labda-cyhalothrin. The low residue concentration of lambda-cyhalothrin can also be attributed to its shorter half-life compared to Cypermethrin. This means that it degrades faster in the vegetables or on the surface than Cypermethrin hence the low concentration in them.

The maximum residue levels for Cypermethrin in collards, swiss chard and tomatoes are 1mg/kg, 2mg/kg and 0.2mg/kg respectively (FAO/WHO, 2009). Collard greens from Soko-mjinga (1.397±0.478mg/kg), swiss chard from Soko-mjinga (2.458±0.298mg/kg) and Ponda-mali (2.495±0.609mg/kg) and tomatoes from all the three markets exceeded the recommended level. Swiss chard from the two markets were 1.229 and 1.248 times higher than the recommended level respectively. Tomatoes from Soko-mjinga, Ponda-mali and Main Municipal Market markets had residues levels that were 1.16, 1.48 and 2.005 times higher the recommended level respectively.

The recommended residues levels for Lambda-Cyhalothrin is 0.3mg/kg in the vegetables (FAO/WHO, 2009). Collard greens from Ponda-mali (0.341±0.164mg/kg) and swiss chard from Soko-mjinga (0.352±0.193mg/kg) exceeded the recommended levels. This shows that consumers are likely to suffer negative chronic health effects due to exposure to the pesticide. Tomatoes from Soko-mjinga and Ponda-mali had the least residue levels of 0.081±0.037 and 0.046±0.049 respectively. This is 3.703 and 6.522 times lower than the recommended residue level respectively.

Conventionally grown Swiss chard had the highest concentration of both pesticides than collard greens and tomatoes. This is because, swiss chard plants are shorter than collard green plants hence if intercropped, the leaves hence less sunlight reaches swiss chard leaves. This reduces the rate of photodegradation of pesticides on its leaves. In addition, swiss chard leaves are dull and wrinkled compared to collard green leaves that are shiny and smooth. This makes it difficult for pesticides to wash off from swiss chard leaves even when it rains. On the other hand, conventional tomato fruits hand the lowest concentration of both pesticides. This can be attributed to its smooth surface that makes it easy to wash off the pesticides when it rains. In addition, farmers use less of the two pesticides on tomatoes since during analysis, other pesticides that were not of interest had higher peaks.

Exposure to high doses of synthetic Pyrethroids such as Lambda-cyhalothrin and Cypermethrin affect the nervous system. This is because they act on sodium channels of nerve cell axons leading to hyperexcitation (Macan *et al.,* 2006 & Bradberry *et al.,* 2005). Other health effects include uncoordination, whole-body tremors, chloreoathetosis and seizures (Ray *et al*., 2000).

In conclusion, organic vegetable samples are safer than conventionally grown vegetables as the concentration of pesticide residues were below detectable levels. Consumers of conventional vegetables are exposed to pesticide residues which can cause negative health effects.

It is recommended that farmers should be encouraged to practice organic agriculture to safeguard their health as well as those of consumers as it reduces exposures to pesticides.

Acknowledgement

We would like to acknowledge Egerton University Alumni Association and Ecological Organic Agriculture Initiative, Egerton University for their support.

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