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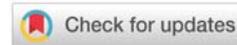
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**\*Corresponding author:** Kingsley George Otchere, Kuapa Kokoo Cooperative Cocoa Farmers and Marketing Union Limited, P. O. Box AN 23044 Asokwa, Kumasi, Ghana., Email: [kgotchere@gmail.com](mailto:kgotchere@gmail.com); [kgotchere@outlook.com](mailto:kgotchere@outlook.com)

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## Research Article

# Analysis of insecticide residues in cabbage (*Brassica oleracea* var. *Capitata*) from three major markets in Kumasi

Kingsley George Otchere<sup>1\*</sup>, Joseph Issifu Adam<sup>2</sup>, John Asiedu Larbi<sup>2</sup>, Sally Amponsah Basil<sup>3</sup> and Albert Banunle<sup>2</sup>

<sup>1</sup>Kuapa Kokoo Cooperative Cocoa Farmers and Marketing Union Limited, Kumasi, Ghana

<sup>2</sup>Department of Theoretical and Applied Biology, KNUST, Kumasi, Ghana

<sup>3</sup>Veolia Ghana Limited, Obuasi, Ghana

## Abstract

Cabbage from 3 major markets (Abinchi, Bantama and Sofoline) in Kumasi were screened for organochlorine, organophosphate and pyrethroid insecticide residues. Ninety cabbage heads - 30 from each market - were randomly sampled and analysed at the Pesticide Residues Laboratories of the Ghana Standards Authority, Accra. The analysis was carried out using Multiple Reaction Monitoring by Gas Chromatography-Pulsed Flame Photometric Detector (GC-PFPD), Gas Chromatography-Electron Capture Detector (GC-ECD) and Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method to detect residues. The limit of detection (LOD) of organochlorine was set at 0.005 mg/kg and that of organophosphate and pyrethroid was set at 0.01 mg/kg. We observed that no organochlorine was present in any of the samples. Only Diazinon was present in the samples from Abinchi and Bantama but at a level ( $0.003 \pm 0.006$  mg/kg) below the maximum residue limit (MRL) of 0.01 mg/kg for cabbage. However, there was a multi-residue contamination in samples from the Sofoline market with Diazinon ( $0.057 \pm 0.098$  mg/kg), Cypermethrin ( $0.007 \pm 0.012$  mg/kg), Fenvalerate ( $0.010 \pm 0.017$  mg/kg) and Lambda-cyhalothrin ( $0.003 \pm 0.006$  mg/kg). With these, only the Diazinon was found to exceed its established MRL (0.01 mg/kg) and so is more likely to pose danger to consumers health.

## Introduction

Cabbage (*Brassica oleracea* var. *capitata*) is recognised as a vital component of human diets by virtue of its high nutritive value, and high content of essential vitamins, proteins, carbohydrates and vital minerals [1]. In addition to its contribution to food needs, cabbage serves as a source of income for people who engage in its production thereby serving as a source of livelihood. According to Drechsel and Keraita (2014) [2] cabbage production serves as a source of income for about 316 urban farmers within the Kumasi metropolis.

As a popular vegetable in urban and peri-urban Ghana, cabbage is mostly attacked by insect pests that lead to reduction in market value and total crop failure. The most serious pests of cabbage are caterpillars of diamond back moth (*Plutella xylostella*), cabbage web worm (*Hellula undulasis*) and

cabbage aphids (*Brevicoryne brassicae*). Susceptibility of cabbage to pests and diseases leads to intensive pest management in its production by farmers. To reduce insect pests' damage, farmers commonly apply synthetic pesticides at different stages of the crop growth [3,4].

Nevertheless, many farmers misapply chemical pesticides by way of applying non-recommended pesticides or applying excess or harvesting shortly after spraying without waiting for the recommended period thereby leaving harmful chemicals residues on them [5-8].

Insecticides are known to result to health hazards including death as a result of accident during application or operation, movement from fields and or residual contamination of food produce and water [9-12]. They are poisonous to nervous system of mammals. Organochlorine pesticides act as nerve



poison by interfering with the transmission of nerve impulse along the axon in poisoned mammals. Organophosphates pose toxic effect on the nervous system by inhibiting the enzyme acetylcholinesterase (AChE) responsible for metabolism of the neurotransmitter Acetylcholine. They are known to cause respiratory problems, memory disorder, neurological deficits, miscarriage, birth defects and cancer. Also, pyrethroid pesticide poisoning in mammals is known to stimulate nerves discharge repeatedly causing hyper-excite.

With knowledge or perception about this, many consumers are sceptical to consume vegetables produced conventionally. Thus, to enhance consumer confidence it is important to monitor and control the residue levels of chemicals used in crop production. The aim of this study was to screen cabbage sold in Abinchi, Bantama and Sofoline markets in the Kumasi Metropolis for the presence and levels of organochlorine, organophosphate and pyrethroid insecticide.

Analysis of pesticide residue levels in food is a relevant mechanism in food quality and safety management by identifying residues levels that can pose human health problems. Previous studies in Ghana, have revealed awkward findings about pesticide residue levels in different food products, and have therefore cautioned for regular monitoring of dietary intake of pesticides residues and their potential health risk.

A study by [13], revealed that some fruit samples contained insecticides residues that are above the accepted Maximum Residue Limit (MRL) which could result in deadly chronic

effects [14]. Also detected that some fruit and vegetable samples collected from major markets across the Greater Accra, Eastern and Central Regions of Ghana contained pesticide residues levels above their Maximum Residue Limits.

As earlier studies have contributed immensely to residue analysis in vegetables particularly cabbage, focus on these three major markets (Abinchi, Bantama and Sofoline) have been limited. Also, as campaign against pesticides misapplication has been on the rise coupled with keen regulation on the production, supply and distribution of pesticides it is important to determine if things have changed with regards to pesticide use on crops and a surest means of detecting this is to screen for pesticides contamination.

## Materials and methods

A total of 90 cabbage heads were randomly sampled from the three markets (Figure 1) on three different occasions over a year i.e. 05-11-2016; 06-13-2017 & 06-27-2017. On each occasion, 10 cabbage heads were picked from each market, making a total of 30 in all, put into separate polythene bags, sealed and appropriately labelled, kept in an ice chest and then transported to the Pesticide Residue Laboratories of the Ghana Standards Authority (GSA) in Accra for the laboratory analysis.

Samples from each market were separately washed properly under running tap water to clean them, as commonly done by consumers, and then chopped separately into pieces and homogenized using FOSS homogenizer (2096). 10 g of each homogenized sample was put in a centrifuge tube, and 10 ml of cold distilled water added to make it paste.

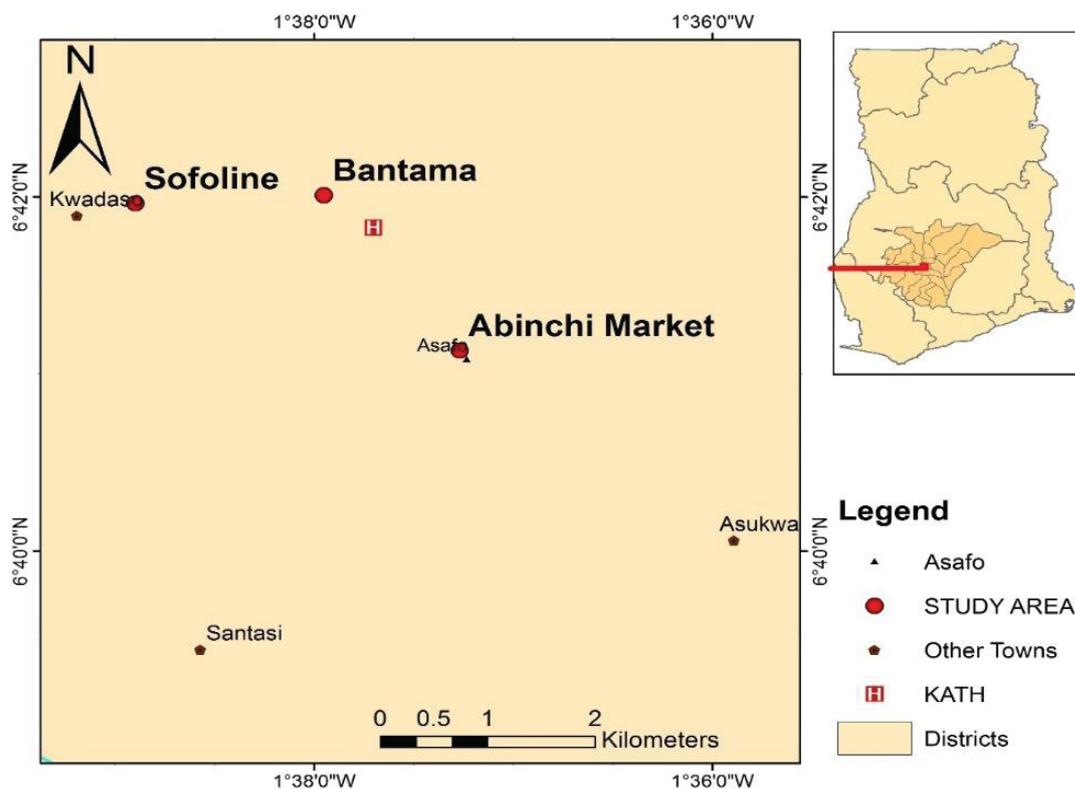


Figure 1: Map showing sampling markets.



The content was vortexed for 30 s to mix it thoroughly. 10 ml of acetonitrile was then added as an extraction solvent and vortexed for 60 s. After, 0.5 g of sodium hydrogencitrate sesquihydrate; 1 g of tri-sodium citrate dehydrate GR; 1 g of sodium chloride CP, and 4 g of magnesium sulphate anhydrous powder were added to the mixture as extraction salts and vortexed for 60 s to obtain the extract. The sodium hydrogencitrate sesquihydrate and tri-sodium citrate dehydrate GR acted as buffer; the sodium chloride CP helped in partitioning the organic and inorganic layers, and the magnesium sulphate anhydrous powder absorbed moisture from the extract.

The extract was then centrifuged at 3000 rpm for 300 s for the organic and inorganic layers to separate. 6 ml of organic layer of the extract was measured into a centrifuge tube. 900 ml of magnesium sulphate anhydrous powder plus 150 mg of PSA (primary and secondary amine) were added to the 6 ml extract content in the centrifuge tube to get rid of residual water and unwanted contaminants in the extract and vortexed for 30 s. The extract was then centrifuged at 3000 rpm for 300 s to obtain a clean extract.

4 ml of the content was pipetted into a 50 ml pear-shape flask, and 40 µl of 5% formic acid prepared in acetonitrile was added to it and taken to a rotary evaporator to concentrate the insecticides i.e. dry out extract to leave insecticides in the flask. The formic acid was added so that the storage stability of the pesticides could be enhanced. After, 1 ml of ethyl acetate was added to dissolve the dried insecticide in the pear-shape flask.

The content was then poured into 2 ml GC vial, and assayed for multi-residues of organochlorine, organophosphate and pyrethroids. A multiple reaction monitoring by GC-PFPD, GC-ECD and QuEChERS method was employed for detection of residues. GSA-prepared standards for the various pesticides were used. The LOD of organochlorine was set at 0.005 mg/kg, and that of organophosphate and pyrethroids was set at 0.01 mg/kg.

The organochlorine and synthetic pyrethroid residues were analysed by CTC ANALYTICS GC machine (CTC01019) equipped with <sup>63</sup>Ni electron capture detector (ECD). The GC conditions used for the analysis were: capillary column coated with VF-5 ms (30 m + 10 EZ Guard, 0.25 mm, 0.25 µm film thickness). Carrier gas and make-up gas was nitrogen at a flow rate of 1 and 29 mL/min, respectively. The temperature of injector operating in splitless mode was held at 270 °C, and that of the electron capture detector was set at 300 °C. The column oven temperature was programmed as follows; 70 °C for 2 min. and increased steadily at a rate of 25 °C/min to 180 °C/min and increased at 5 °C/min up to 300 °C. The injection volume of the GC was 1 µL.

The organophosphorus residues were analysed by same machine make equipped with pulse flame photometric detector (PFPD). The GC conditions used for the analysis were: capillary column coated with VF-1701 ms (30 m, 0.25 mm, 0.25 µm film thicknesses). Carrier gas was nitrogen at a flow rate of 2 mL/min with Air 1, Air 2 and H<sub>2</sub> flow rates of 17, 10 and 14 mL/min

respectively. The temperature of injector operating in splitless mode was held at 270 °C and the PFPD was set at 280 °C. The column oven temperature was programmed as follows; 70 °C for 2 min. and increased steadily at a rate of 25 °C/min to 200 °C/min and increased at 20 °C/min up to 250 °C.

Extracts of fortified samples were serially diluted by a factor of 2 to give different concentrations. 1 µl of each concentration was injected and the least concentration that gave response was noted, and LOD was calculated by the formula:

$$\text{LOD} = (V_1/V_2) * \text{concentration fortified}$$

$V_1$  – volume injected

$V_2$  – final volume of fortified extract

The detection limit for organochlorine pesticides was determining to be 0.005 mg/kg, while that of both organophosphorus and pyrethroid pesticides were 0.01 mg/kg.

## Results

Fifteen organochlorine including Aldrin, Alpha-endosulfan, Beta-Endosulfan, Beta-HCH, Delta-HCH, Dieldrin, Endosulfan Sulphate, Endrin, Gamma-chlordane, Heptachlor, Lindane, Methoxychlor, P'P DDE, P'P-DDD and P'P-DDT were screened for. However, none of them was detected in any of the samples.

Of the 13 organophosphates screened for i.e. Chlorfenvinphos, Chlorpyrifos, Diazinon, Dimethoate, Ethoprophos, Fenitrothion, Fonofos, Malathion, Methamidophos, Parathion, Phorate, Pirimiphos-methyl and Profenofos, only Diazinon was detected at different concentrations in the different market samples. In both Abinchi and Bantama markets, the concentration of Diazinon was (0.003 ± 0.006 mg/kg) which is below its EU MRL of 0.01 mg/kg for cabbage. However, in the Sofoline market the concentration of Diazinon was 0.057 ± 0.098 mg/kg, which is higher than its MRL (Table 1).

Nine pyrethroids including Allethrin, Bifenthrin, Cyfluthrin,

**Table 1:** The results of organophosphate insecticide analysis from cabbage.

Insecticide	Abinchi	Bantama	Sofoline	EU MRL
	Mean ± SD	Mean ± SD	Mean ± SD	
Chlorfenvinphos	Nd	Nd	Nd	0.01 (Max)
Chlorpyrifos	Nd	Nd	Nd	1.00 (Max)
Diazinon	0.003 ± 0.006	0.003 ± 0.006	<b>0.057 ± 0.098</b>	0.01 (Max)
Dimethoate	Nd	Nd	Nd	0.02 (Max)
Ethoprophos	Nd	Nd	Nd	0.02 (Max)
Fenitrothion	Nd	Nd	Nd	0.01 (Max)
Fonofos	Nd	Nd	Nd	0.01 (LOD)
Malathion	Nd	Nd	Nd	0.02 (Max)
Methamidophos	Nd	Nd	Nd	0.01 (Max)
Parathion	Nd	Nd	Nd	0.05 (Max)
Phorate	Nd	Nd	Nd	0.01 (Max)
Pirimiphos-methyl	Nd	Nd	Nd	0.05 (Max)
Profenofos	Nd	Nd	Nd	0.01 (Max)



Cypermethrin, Deltamethrin, Fenpropathrin, Fenvalerate, Lambda-cyhalothrin and Permethrin were screened for. With these, only 3 namely Cypermethrin, Fenvalerate and Lambda-cyhalothrin were detected, only in samples from the Sofoline market, at concentrations of  $0.007 \pm 0.012$  mg/kg;  $0.010 \pm 0.017$  mg/kg and  $0.003 \pm 0.006$  mg/kg respectively (Table 2). These concentrations were below the respective established MRLs for cabbage (i.e. 1.00 mg/kg for Cypermethrin and 0.02 mg/kg for Fenvalerate and Lambda-cyhalothrin) and so are not likely to pose health danger when consumed.

**Table 2:** The results of pyrethroid insecticide analysis from cabbage.

Insecticide	Abinchi Mean $\pm$ SD	Bantama Mean $\pm$ SD	Sofoline Mean $\pm$ SD	EU MRL
Allethrin	Nd	Nd	Nd	0.01 (LOD)
Bifenthrin	Nd	Nd	Nd	1.00 (Max)
Cyfluthrin	Nd	Nd	Nd	0.30 (Max)
Cypermethrin	Nd	Nd	$0.007 \pm 0.012$	1.00 (Max)
Deltamethrin	Nd	Nd	Nd	0.01 (Max)
Fenpropathrin	Nd	Nd	Nd	0.01 (Max)
Fenvalerate	Nd	Nd	$0.010 \pm 0.017$	0.02 (Max)
Lambda-cyhalothrin	Nd	Nd	$0.003 \pm 0.006$	0.02 (Max)
Permethrin	Nd	Nd	Nd	1.05 (Max)

## Discussion

The absence of organochlorine pesticides in our analysis might be because the farmers did not apply any organochlorine insecticide as this category of pesticides have been banned since 1994 [15] or organochlorines were present but at levels less than their LOD of 0.005 mg/kg. A study by [16] reported that cabbage samples together with other vegetables and fruits purchased from some markets in the Kumasi metropolis including the Bantama market contained organochlorine pesticides such as Gamma-HCH, Methoxychlor, Dieldrin, Endrin, p,p'-DDE and p,p'-DDT. [17] also found that cabbage samples from the Ejisu-Juaben Municipal, a major source of cabbage to markets in the Kumasi metropolis contained residues of Alpha BHC, Gamma BHC (Lindane), Delta BHC, Heptachlor, Aldrin, DDT, DDE, Endrin, Beta Endosulfan, Dieldrin, Endosulfan sulphate and Beta BHC at concentrations greater than their MRLs. But our finding contrasts these findings.

Our finding suggests that the farmers never used any organochlorine on their farms. Even if they used, it was before the period 1994 when this category of insecticides was banned in Ghana, and so it was possible to detect them some years after their ban due to bioaccumulation or residues that persisted might have been remediated over time and so would not be present for detection in recent times.

The very high concentration of Diazinon in the Sofoline samples might be as a result of producers for the market not complying with the prescribed dosage of the insecticide or the waiting time before harvesting their cabbage for sale or both. According to [5,6] some cabbage farmers apply insecticides

in overdose to improve their efficacy in controlling insects or continue to spray produce immediately before they harvest them to the market so that the produce will look appealing to buyers. Disregarding prescribed dosage and waiting period can make residues persist on produce and render them hazardous to consumers.

The presence of multi-residues of pyrethroids in the Sofoline samples might be because producers for the market used a mixture of different pyrethroid insecticides to control insects on their crops but failed to comply with their prescribed waiting periods. According to [17], majority of farmers use a mixture of two or more pesticides by relying on trade names without consideration of compatibility or active ingredients.

## Conclusion

The cabbage heads sampled from Abinchi and Bantama were found to contain only Diazinon and at a safe level. However, those from Sofoline contained multi-residues including Diazinon, Cypermethrin, Fenvalerate and Lambda-cyhalothrin. With these, Diazinon exceeded its MRL. The results suggest that some cabbage farmers still misapply some approved insecticides and so we advocate that farmer education on insecticides or pesticides use and safety should be intensified especially for small scale vegetable farmers. We again advocate that policies or programmes that seek to motivate vegetable farmer (or all farmers) to comply with insecticides or pesticides use guidelines should be put in place by the Government or agriculture related institutions. In this regard, we suggest 'residue-free award schemes' to be featured in the national farmers day celebration to encourage compliance.

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