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Evaluation of pesticide residues in tomato (*Lycopersicum esculentum*) and the potential health risk to consumers in urban areas of Ghana

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ABSTRACT: Kumasi, Sekondi-Takoradi, and Accra are the major recipients of agricultural commodities or productions in Ghana, primarily due to their population and ready markets. To ensure food security, meet food demands, and mitigate the threats posed by pests and diseases, pesticides are used when cultivating vegetables and fruits in Ghana. The present study has been conducted to assess the concentration of various pesticide residues in tomatoes, obtained from three market centers (namely Bantama, Central, and Ayigya Markets) in Kumasi, dealing with potential health risks for the consumers. Analyzed samples have revealed high percentage of organophosphorous pesticide residues (45%) in Ayigya market with Bantama market recording high percentage of organochlorines and pyrethroid. Laboratory analysis of tomato samples for various pesticides residues has indicated that all the pesticide residues pose no threat to human's health with all estimated hazard indices being below 1; however, heptachlor (HI=0.85) and dieldrin (HI=0.74) have shown the highest risk levels in children. The study reveals that there is some need for strict monitoring of heptachlor and dieldrin in tomato, especially in case of children.

Keywords: hazard index, organophosphorous organochlorines, pesticide residues, pyrethroid.

INTRODUCTION

Agriculture has been the mainstay of Ghanaian economy for some decades before the discovery of oil in 2007. In 2005, it contributed the most to Ghana's economy, accounting for 35% of GDP and 36% of export earnings, and employing 60% of the Ghana labor force (Asare, 2012). Despite the hazardous effect of pesticides, farmers continue using agrochemicals to enhance agricultural productivity and provide adequate food supplies for the increasing population, thus ensuring food security. Pesticides are compounds with known inherent toxicity. Their heavy application result in some pollution that might disturb natural balance, leading to wide spread of pest resistance as well as environmental pollution and hazards to both humans and

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wildlife (Claeys et al., 2011; Hjorth et al., 2011). It has been established that exposure to pesticides through one's diet is more prevalent than other ways, such as air and drinking water (Claeys et al., 2011; Chen et al., 2011).

In Ghana, chemical pesticides are used extensively in both subsistence and commercial farming. In the cocoa sector, the use of pesticides in 2012 helped the country produce over one million metric tons of cocoa, an unprecedented production capacity in the history of Ghana. A similar success rate has also been observed for cashew nut (Asare, 2012; Gain Report, 2012). Organochlorines, carbamates, organophosphates, and pyrethriods are the most commonly-applied pesticides and insecticides to control pest and eradicate disease vector control in most developing countries, like Ghana (Ghana EPA, 2007). It has been estimated that 87% of farmers in Ghana use chemical pesticides to control pests and diseases, threatening vegetables and fruits (Akoto et al., 2013). The organophosphates affect nervous system by inhibiting cholinesterase, an enzyme that regulates the neurotransmitter acetylcholine. They are known to affect neuro-development too. Similarly, both carbamates and pyrethriods, the synthetic version of the naturally-occurring pesticide pyrethrin, are toxic to the nervous system as well. However, there is widespread concern about how exposure to pesticides might affect children, who are likely to be vulnerable to the toxic effects of pesticides the most. Children drink more water, eat more food, and breathe more air than adults; therefore, they receive substantially heavier exposure to any toxicants in water, food, or air than adults (Suk et al., 2003; Kimmel et al., 2005; Bradman et al., 2007; Bempah et al., 2011; Akoto et al., 2013).

Due to the adverse effect of these pesticides on the environment and human life, their production and use have been severely restricted and at times banned altogether, especially organochlorine compounds, which are the most extensively-used agrochemical by farmers in both developed and industrialized countries. Many researchers have documented the toxicological effects of these compounds on people, exposed to them. Exposure to these contaminants occurs through ingestion, inhalation, and dermal contact. Generally, food is the main way of exposure as it is assumed to be five times greater than other ways, like air and drinking water (Juraske et al., 2009). Accordingly, many study have focused on the effect of high pesticide exposure to Ghanaian farmers, especially vegetable farmers (Ntow et al., 2006; Amoah et al., 2006; Ntow et al., 2008; Dinham, 2003; Darko and Akoto, 2008; Armah, 2011; Bempah et al., 2011).

Ntow (2001) noted that 87% of urban and semi-urban vegetable farmers use highly synthetic persistent organochlorines such as lindane, endosulfan, and DDT to control pests and illnesses on vegetables and fruits. Although Act 528, established in 1999 (Provisional list of Banned Pesticides in Ghana), banned the use of DDT in Ghana, the chemical is still a predominant environmental contaminant in the Ghanaian environment.

Another study, conducted by Daniel et al. (2008), also revealed that for farmers the main factor to determine the type of used pesticide included the level of efficacy, availability, affordability and safety. Yet, most users paid less attention to the regulations (whether banned or restricted) regarding the use of the pesticides. The study further revealed high doses of chlorpyrifos, DDT, cypermethrin, and dimethoate in vegetable samples, subjected to gas chromatography analysis. The present study has assessed and evaluated the health risk in terms of susceptibility and exposure levels to both children and adults from the concentration of pesticide residues in tomato brought to three urban markets. It also evaluated the potential health risk to the consumers.

MATERIALS AND METHODS

Study area

The study has taken place in Kumasi, the capital city of the Ashanti Region, Ghana, as illustrated in Figure 1, where many farmers bring their harvested tomatoes to sell. Kumasi is located in the transitional forest zone of Ghana, about 270 km north of the nation's capital. Being the second largest city in Ghana with an estimated daytime population of about 2 million, the city has become inundated with high numbers of migrants due to its rapid urbanization. The climate is hot and humid with a maximum and minimum average temperature of 30.7^{o}C and 21.5^{o}C respectively. The present study has been carried out in three markets sites located within the center of the city: Bantama Market, Central Market, and Ayigya Market. The city's location with main roads to link it to other parts of the country and as a large recipient of agricultural productions, consumed locally, makes it imperative to control pesticide residues in fruits and vegetables.

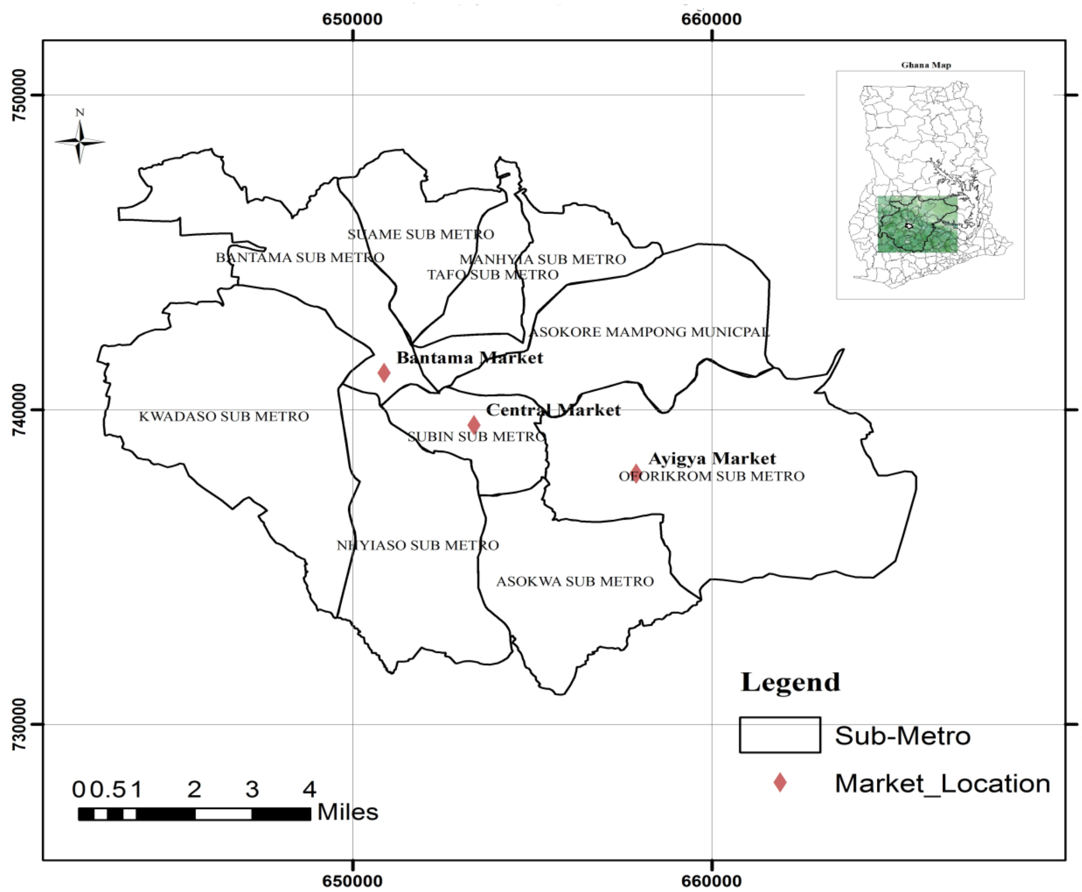

Fig. 1. Map of the various sample sites

Sampling, Preservation and Analysis

All eighteen tomato samples were bought from three market centers, serving as the main points for vegetable supply in the study area. Six different samples were collected from each market to be sealed, labeled, placed in an insulated ice box, and immediately sent to the Ghana Standards Authority Pesticide Residue Laboratory for analysis. The samples were prepared in accordance to Quechers-Mini-Multiresidue Method (QMMM). This was followed by a

Varian gas chromatograph analysis, CP-3800 equipped with ^{63}Ni Electron Capture Detector (ECD) to ascertain the level of pesticides in each sample. Pesticide reference standards, whose certified purity was at least 98%, were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and stored in a freezer at 20^{o}C to minimize degradation. For analysis, the pesticide grade, ethyl acetate, and acetone were supplied by Labscan (Dublin, Ireland), whereas anhydrous sodium, hydrogen carbonate, and sodium sulphate analytical grade were purchased from Merck (Darmstadt, F.R. Germany).

Sample extraction procedure

As aforementioned QMMM was applied to extract the samples. Fresh collected tomato samples were thoroughly shredded and homogenized. Ten grams of macerated tomato sample was weighed and transferred into 50 ml centrifuge tubes, capped, and allowed to mix well in a multi-Shaker over 12 hrs at 150 rpm at about 20-25°C. Ten ml of acetonitrile was added. A buffer salt mixture, consisted of 4.0 g magnesium sulphate anhydrous grit, 1.0 g sodium chloride, 0.5 g disodium hydrogen citrate sesquihydrate, and 1.0 g tri-sodium citrate dehydrate, was added to induce phase separation and pesticide partitioning. It was subsequently shaken vigorously for one minute. The samples were then centrifuged for five minute at 3000 rpm. Six ml of the supernatant were transferred into a 15 ml Teflon centrifuge tube, containing 900mg of MgSO4 and 150 mg Primary-Secondary Amine, (PSA), shaken and centrifuged for another five minute at 3000 rpm. Three ml of the supernatant were reconstituted in one ml of ethyl acetate for GC- ECD analysis.

Sample analysis

Aliquots of final volumes were quantified by Varian gas chromatograph CP-3800, equipped with ^{63}Ni ECD and a capillary column, coated with ZB-5 (30m x 0.25, 0.25 µm film thickness). For calibration purposes, 9975 µl ethyl acetate was added to 25 µl of organochlorine mix standard solution (2.0 µg/ml). Serial dilutions of 0.20 µg/ml, 0.02 µg/ml, 0.010 µg/ml, and 0.005 µg/ml concentrations were prepared with 1 µl of each inserted into the injection port of GC-ECD and the response was obtained. The suspected pesticide was identified by comparing peak retention times in samples with the ones in the pure analytical standards. Nitrogen was used as carrier gas at a flow rate of 1.0 ml/min to make up gas of 29 ml/min. The injector and detector temperatures were set at 280^{o}C and 300^{o}C, respectively. The column temperature began at 60°C for two minutes, then 180^{o}C per minute up to 300°C, where it was held for 31.8 min.

Quality assurance procedure

Quality control and quality assurance happened next in order to determine multi residue method's performance. Quantification recoveries and limits were determined on samples at spiking levels of 10-50 µg kg^{-1} from the pesticide mixture standard with the average recovery ranging from 83.5 to 112.5%. Blanks were fortified with mixture and analyzed as a normal sample with each set of samples. Accordingly, the sample analysis data were corrected for these recoveries.

Health risk assessment

Health risk indices were estimated based on both pesticide residues, detected in tomatoes, and exposure assumptions. The adopted health risk assessment of dietary pesticide intake in fruits was based on US Environmental Protection Agency's guideline for a hypothetical body weight of 10 kg for children and 70 kg for adults. Both maximum absorption rate and bioavailability rate were 100% (US-EPA, 1989, 1996). It was assumed that the quantity of tomato consumed by an average person in a day was 0.037 kg/day (International Food Policy Research Institute, 2004; FAO, 2015). Maximum

Residue Level (MRL) is the maximum concentration of pesticide residue, in mg/kg, which is legally permitted in food commodities and animal feeds. Acceptable Daily Intake (ADI) or reference dose of a chemical as defined by the FAO is the daily intake which, during an entire lifetime. It seems to be without any appreciable risk to the consumer's health (FAO/WHO, 2015). In this study, for each type of pesticide residue detected, the estimated lifetime exposure dose (mg/kg/day) (EADI) was obtained by multiplying the residual pesticide concentration (mg/kg) in the studied food (in this case tomato) by the food consumption rate (kg/day), and divided by the body weight (kg) as shown in Equation (1). The hazard indices (HI) for adults and children were calculated as ratios between estimated pesticide exposure doses and the reference doses, considered to be safe levels of exposure over a lifetime as shown in Equation (2) (US-EPA, 1989, 1996).

$$EADI = \frac{Residual\ pesticide\ concentration(mg/kg)\ X\ Consumption\ rate(kg/day)}{Body\ weight(kg)} \quad (1)$$

$$\text{Hazard Index (HI)} = \frac{\text{EADI}\,(\text{mg kg}^{-1}\text{day}^{-1})}{\text{Reference dose}\,(\text{mg kg}^{-1}\text{day}^{-1})} \quad (2)$$

RESULTS AND DISCUSSION

Pesticide residue analysis

Figure 2 presents the comparison between the percentage of each group of pesticides contributes to the total concentration in sampling sites. Although all the pesticides contributed quite equally to the total concentrations, samples obtained from Bantama Market recorded the highest levels of organchlorines and pyrethroid, whereas high levels of organophosphorous belonged to Ayigya market.

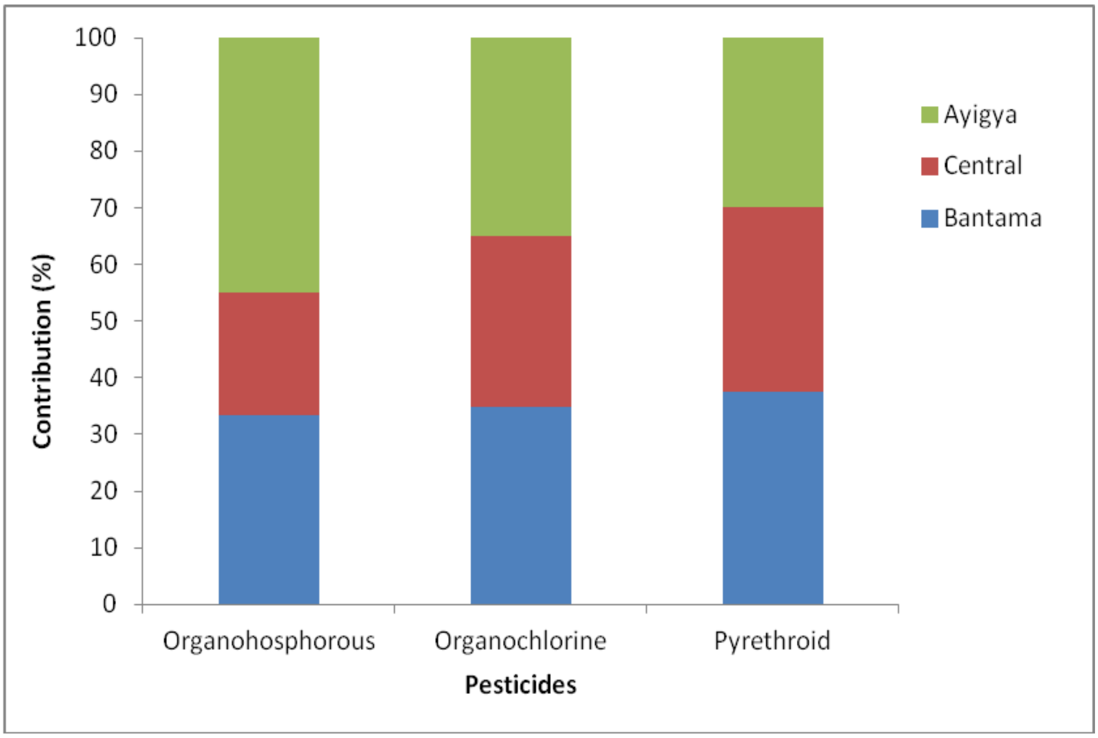

Fig. 2. **Percentage contributions of pesticides based on their concentrations in sampling points**

Figures 3, 4, and 5 illustrate the results of pesticide residues, detected and quantified in tomato for organophosphorous, organchlorines and pyrethroid pesticide residues respectively. In all three markets levels of organophosphorous pesticides levels varied from 0.001 to 0.031 mg/kg and as for organochlorines and pyrethroid these rates changed to 0.001-0.015 mg/kg and 0.001-0.020 mg/kg respectively. Out of the 13 organophosphorous pesticides, analysed, chlorpyrifos, chlorfenvinphos, pirimiphos-methyl, dimethoate, profenofos, and fonofos were detected in all samples, obtained from the three markets, with chlorpyrifos (0.031 mg/kg being the highest in Bantama market, and diazinon and pirimiphos-methyl (0.001 mg/kg) the lowest detected chemicals in all three market centers; however, none of the samples from the three markets was found to be contaminated with methamidophos (Fig. 3). Additionally, dimethoate was detected as the highest both in Central and Ayigya markets. Parathion-methyl, malathion, and phorate were detected only in samples, obtained from Ayigya market. In case of organochlorines, the highest residue concentration level was detected by endosulfan-sulfate (0.015 mg/kg) from Bantama Market without any α-endosulfan, aldrin, γ-chlordane, and β-endosulfan detected in samples from either Bantama or Ayigya Markets in addition to allethrin and methoxychlor from Bantama market and p,p'–DDE from Ayigya market (Fig. 4). The concentration of the various organochlroine pesticides analyzed in this study is presented in Figure 4. All samples from Central markets were found to be contaminated with all organochlorine chemicals, analyzed in this study with the exception of α-endosulfan, aldrin, methoxychlor, γ-chlordane, and β-endosulfan. Nevertheless, samples from Central and Ayigya Markets were found to be contaminated with heptachlor at higher concentration levels of 0.012 mg/kg and 0.013 mg/kg respectively. Samples from Bantama and Central Markets showed same concentration level of 0.001 mg/kg for p,p'–DDE as the lowest while methoxychlor (0.001 mg/kg) was detected as the lowest in Ayigya market.

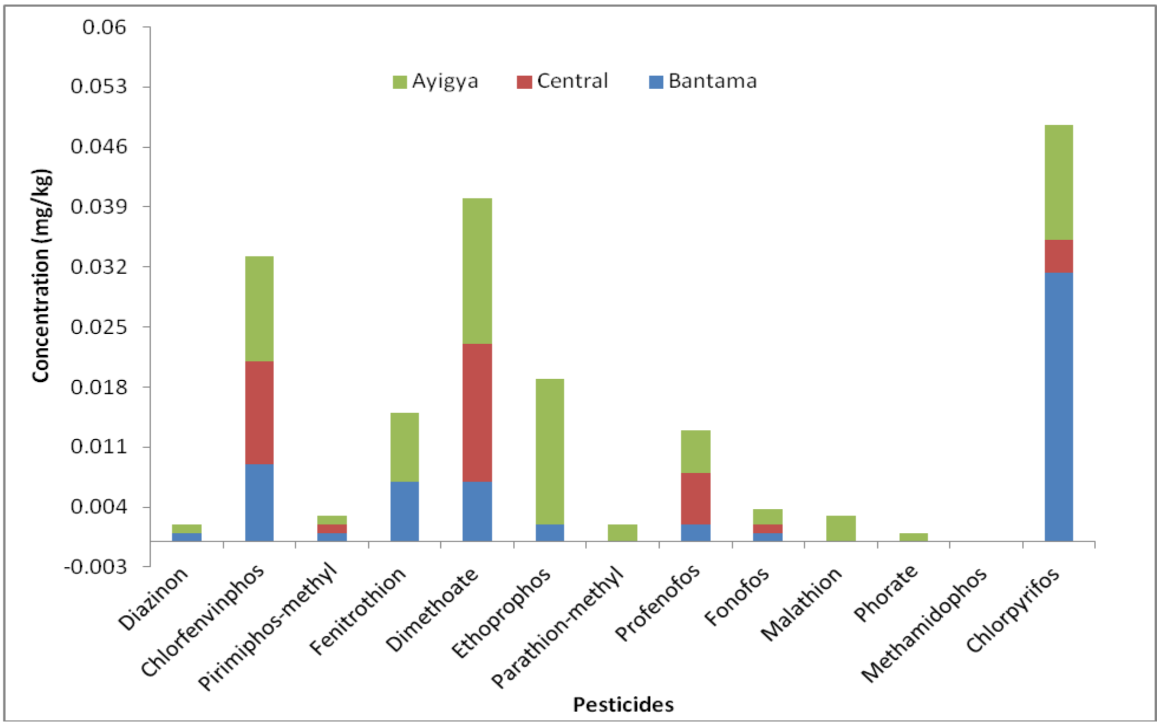

Fig. 3. Average concentration of detected organophosphorous pesticide residues

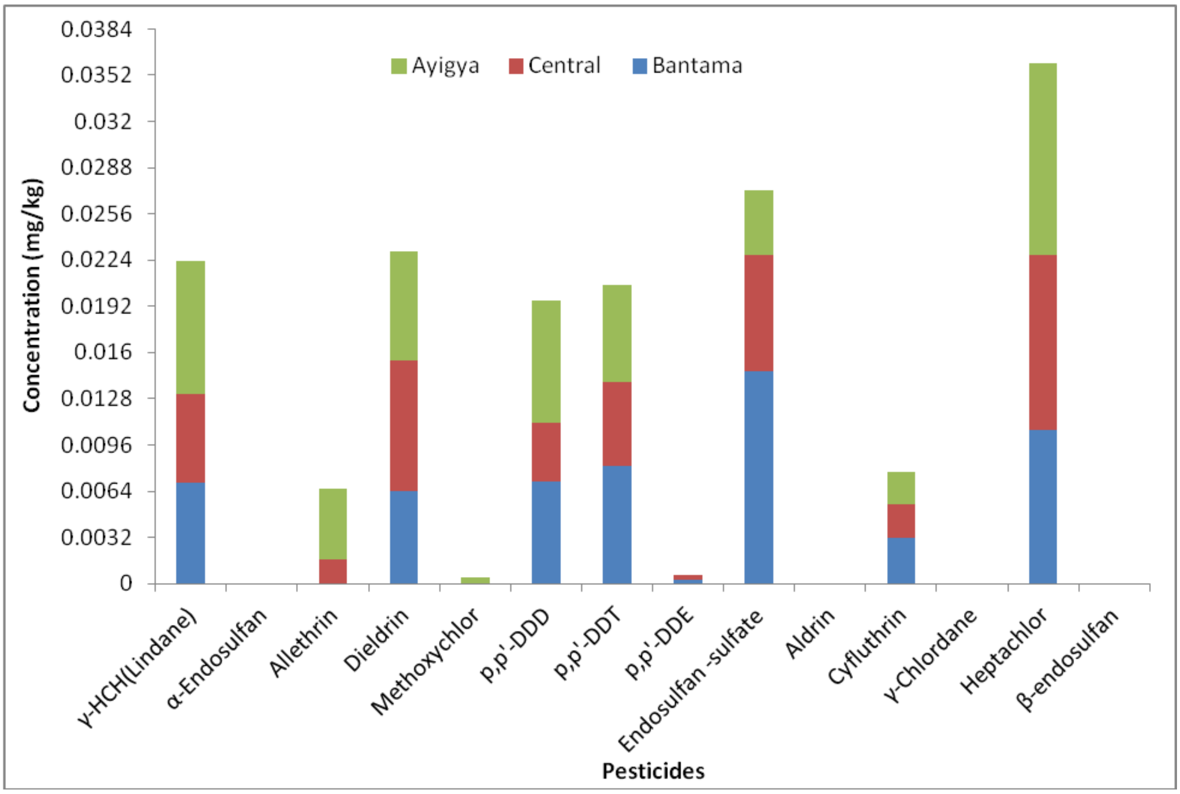

Fig. 4. Average concentration of detected organochlorine pesticide residues

Among pyrethroid pesticides, bifenthrin, deltamethrin, λ-cyhalothrin, fenpropathrin, permethrin, cypermethrin, and fenvalerate were the only chemicals detected. Figure 5 presents the concentration of detected pyrethroid pesticides. Deltamethrin had the highest concentration level of 0.020 mg/kg for Bantama and 0.017 mg/kg for both Central and Ayigya Markets. Notwit standing, λ-cyhalothrin was not detected in samples from Ayigya. Again, the highest and lowest pyrethroid pesticide residue was detected in samples from Bantama market. Overall, the results in this study established high levels of organophosphourous, organochlorine, and pyrethroid pesticides in samples from Bantama market compared to the other two, indicating a great potential concern for food items from this market, despite the fact that the residues level were below the established maximum residue level (MRL) guidelines values of FAO or WHO. Yet, the regular consumption of tomato with modest contamination can result in accumulation in the consumer's body with its attending health problems in the long run, particularly to the most vulnerable population sub-group, i.e. the children. The good news is that the results from this study reveal a lower pesticides residue levels, compared to the previous studies by Nakata et al. (2002) in China, Adeyeye and Osibanjo (1999) in Nigeria, by Bempah et al. (2011) in Ghana, and by Bhanti and Taneja (2007) in India.

Health risk estimates

Tomato samples were analyzed for 34 residues, comprising 13 organophosphorous, 14 organochlorines, and 7 synthetic pyrethroid. Mean residual concentration of the various organophposphorous, organochlorines, and pyrethroid pesticides in the samples are given in Tables 1, 2, and 3, respectively.

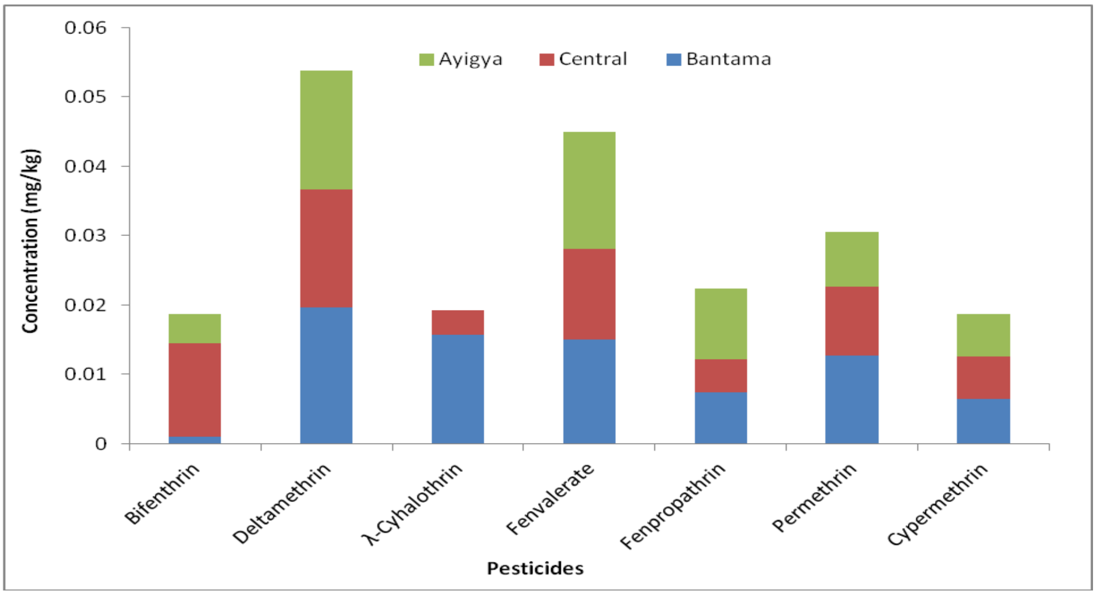

Fig. 5. Average concentration of pyrethroid pesticide residues detected in tomato

Table 1. Mean concentration of detected organophosphorous pesticide residues

Pesticides	Bantama	Central	Ayigya
Chlorpyrifos	0.031 ± 0.032	0.004 ± 0.002	0.013 ± 0.018
Chlorfenvinphos	0.009 ± 0.000	0.012 ± 0.000	0.012 ± 0.005
Fenitrothion	0.007 ± 0.000	ND	0.012 ± 0.000
Diazinon	0.001 ± 0.000	ND	0.001 ± 0.000
Pirimiphos-methyl	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Dimethoate	0.007 ± 0.000	0.016 ± 0.000	0.017 ± 0.016
Ethoprophos	0.002 ± 0.000	ND	0.002 ± 0.001
Fonofos	0.001 ± 0.000	0.001 ± 0.000	0.002 ± 0.001
Profenofos	0.002 ± 0.000	0.006 ± 0.000	0.005 ± 0.003
Malathion	ND	ND	0.003 ± 0.001
Methamidophos	ND	ND	ND
Parathion-methyl	ND	ND	0.002 ± 0.000
Phorate	ND	ND	0.001 ± 0.000

ND = Not Detected

Table 2. Mean concentration of detected organochlorine pesticide residues

Pesticides	Bantama	Central	Ayigya
γ-HCH(Lindane)	0.007 ± 0.003	0.006 ± 0.001	0.009 ± 0.002
α-Endosulfan	ND	ND	ND
Allethrin	ND	0.002 ± 0.002	0.005 ± 0.005
Dieldrin	0.006 ± 0.003	0.009 ± 0.006	0.008 ± 0.006
Methoxychlor	ND	ND	0.001 ± 0.000
p,p'-DDD	0.007 ± 0.005	0.004 ± 0.003	0.008 ± 0.007
p,p'-DDT	0.008 ± 0.006	0.006 ± 0.007	0.007 ± 0.003
p,p'-DDE	0.001 ± 0.000	0.001 ± 0.000	ND
Endosulfan-sulfate	0.015 ± 0.012	0.008 ± 0.007	0.005 ± 0.002
Aldrin	ND	ND	ND
Cyfluthrin	0.003 ± 0.001	0.002 ± 0.001	0.002 ± 0.000
γ-Chlordane	ND	ND	ND
Heptachlor	0.011 ± 0.003	0.012 ± 0.007	0.013 ± 0.006
β-endosulfan	ND	ND	ND

ND = Not Detected

Table 3. Mean concentration of detected pyrethroid pesticide residues

Pesticides	Bantama	Central	Ayigya
Bifenthrin	0.001 ± 0.001	0.014 ± 0.023	0.004 ± 0.004
Deltamethrin	0.020 ± 0.006	0.017 ± 0.009	0.017 ± 0.003
λ-Cyhalothrin	0.016 ± 0.011	0.004 ± 0.003	ND
Fenvalerate	0.015 ± 0.013	0.013 ± 0.011	0.017 ± 0.000
Fenpropathrin	0.007 ± 0.006	0.005 ± 0.007	0.010 ± 0.010
Permethrin	0.013 ± 0.003	0.010 ± 0.005	0.008 ± 0.002
Cypermethrin	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001

ND = Not Detected

Health risk assessment in the present study was estimated based on pesticides residues, detected in tomato (Tables 4-6). Adopted dietary pesticide intake in vegetables was based on U.S. Environmental Protection Agency's guideline for a hypothetical body weight of 10-kg for children as well as 0.037 kg/person/day as consumption rate for tomato by the International Food Policy Research Institute. To obtain Estimated Average Daily Intake (EADI) the average residual pesticide concentration (mg/kg) was multiplied by the food consumption rate (kg/day), whereas the Hazard Indices (HI) were obtained by dividing ADI (mg/kg/day) into their corresponding values of WHO/FAO acceptable daily intakes (WHO, 1997). For lack of ambiguity and simplicity, the maximum concentration obtained from the three markets was used as a representative sample to estimate the hazard index for both children and adults.

As depicted in Table 4 below, the health risk estimates revealed that the organophosphorous pesticide residues do not pose any direct threat to human's health with all the hazard index under the safe limit (<1), 0.000007-0.021 and 0.00005-0.148 for adult and children respectively. Chlorfenvinphos recorded the highest hazard index (equal to 0.148) for children and malathion, the lowest for adults. Results indicate much potentiality for systemic toxicity in children who are considered to be the most vulnerable population subgroup.

Table 4. Health risk estimation of detected organophosphorous pesticide residue in tomato

Pesticide	Reference dose (mg/kg/day)	Estimated dose (mg/kg/day)		Hazard index	Health risk
		Children	Adults		
Diazinon	0.005	3.70E-06	5.29E-07	0.0003[Adult]	No
				0.0019[Children]	No
Chlorfenvinphos	0.0005	7.40E-05	1.06E-05	0.021[Adult]	No
				0.148[Children]	No
Fenitrothion	0.006	2.96E-05	4.23E-06	0.0009[Adult]	No
				0.006[Children]	No
Dimethoate	0.002	7.40E-05	1.06E-05	0.005[Adult]	No
				0.037[Children]	No
Ethoprophos	0.0004	7.40E-06	1.06E-06	0.003[Adult]	No
				0.019[Children]	No
Parathion-methyl	0.003	7.40E-06	1.06E-06	0.0004[Adult]	No
				0.0025[Children]	No
Fonofos	0.002	1.11E-05	1.59E-06	0.0008[Adult]	No
				0.006[Children]	No
Malathion	0.3	1.48E-05	2.11E-06	0.000007[Adult]	No
				0.00005[Children]	No
Phorate	0.0007	3.70E-06	5.29E-07	0.0008[Adult]	No
				0.0053[Children]	No
Methamidophos	0.004	7.40E-06	1.06E-06	0.0003[Adult]	No
				0.0019[Children]	No
Chlorpyrifos	0.01	2.22E-04	3.17E-05	0.003[Adult]	No
				0.022[Children]	No

Results in Table 5 also indicate that the application of organochlorine pesticides on vegetable do not pose any direct threat to human's health, though there is cause for the use of heptachlor and dieldrin as the hazard index shows an approximate value close to one for children, while the rest of the organochlorine pesticide residues belong to no-risk class. The hazard index of organochlorine pesticide residue in tomato ranged between 0.00007-0.122 and 0.0005-0.851 for adults and children respectively.

Table 6 shows that all the synthetic pyrethroid pesticide residues detected in tomato pose no direct threat to human's health with hazard index recorded within 0.0008-0.0021 and 0.0056-0.014 for adults and children respectively. But the highest hazard index was recorded in bifenthrin for children, with cyhalothrin and fenvalerate recording the lowest for adult. Overall, potential health risk to population might be entailed by lifetime consumption of these vegetables as the indices for heptachlor, dieldrin, and chlorfenvinphos residues were high, especially in children, even though they were theoretically not high enough (>1) to pose health threats.

Table 5. Health risk estimation for organochlorine pesticide residue in tomato

Pesticide	Reference dose (mg/kg/day)	Estimated dose (mg/kg/day)		Hazard index	Health risk
		Adults	Children		
Gamma-HCH(Lindane)	0.005	4.07E-05	5.81E-06	0.0011[Adult]	No
				0.0081[Children]	No
Heptachlor	0.0001	8.51E-05	1.22E-05	0.122[Adult]	No
				0.851[Children]	No*
Allethrin	0.003	2.96E-05	4.23E-06	0.0014[Adult]	No
				0.0099[Children]	No
Dieldrin	0.0001	7.40E-05	1.06E-05	0.105[Adult]	No
				0.74[Children]	No*
Fenpropathrin	0.03	8.14E-05	1.16E-05	0.0004[Adult]	No
				0.0027[Children]	No
Cyfluthrin	0.04	1.85E-05	2.64E-06	0.00007[Adult]	No
				0.0005[Children]	No
Permethrin	0.05	5.92E-05	8.46E-06	0.0002[Adult]	No
				0.0012[Children]	No
p,p'-DDD	0.01	5.55E-05	7.93E-06	0.0008[Adult]	No
				0.0056[Children]	No
p,p'-DDT	0.01	5.92E-05	8.46E-06	0.0008[Adult]	No
				0.0059[Children]	No

*Potential risk

Table 6. Health risk estimation for synthetic pyrethroid pesticide residue in tomato

Pesticide	Reference dose (mg/kg/day)	Estimated dose (mg/kg/day)		Hazard index	Health risk
		Adults	Children		
Bifenthrin	0.01	1.44E-04	2.06E-05	0.0021[Adults]	No
				0.014[Children]	No
Deltamethrin	0.01	1.07E-04	1.53E-05	0.0015[Adults]	No
				0.0107[Children]	No
Lambda-Cyhalothrin	0. 02	1.15E-04	1.64E-05	0.00082[Adults]	No
				0.0057[Children]	No
Fenvalerate	0.02	1.11E-04	1.59E-05	0.0008[Adults]	No
				0.0056[Children]	No

CONCLUSIONS

The analysis revealed that tomato contains pesticide residues with doses below the reference ones. Hazard index for various pesticide residues also revealed an HI value below 1, suggesting that the pesticide residues, present in the tomato incur no threat to human's health, but there should be routine monitoring programs of heptachlor and dieldrin levels especially in children, the most vulnerable population subgroup, which recorded HI values close to 1 in order to control, reduce, and minimize health risks.

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