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International Journal of Current Research Vol. 4, Issue, 12, pp. 455-460, December, 2012 INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ASSESSMENT OF THE LEVELS OF PYRETHROIDS PESTICIDE RESIDUES IN PEPPER (Capsicum annuum L) FROM LIBGA IN THE NORTHERN REGION OF GHANA

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ARTICLE INFO ABSTRACT Pyrethroids are synthetic forms of pyrethrins and that differ in chemical structure and symptoms of Article History: exposure. Some pyrethroids are known to be endocrine destructive, and impair reproductive Received 24th September, 2012 Received in revised form competence. They are used as pesticides in the cultivation of crops and vegetables. Investigations 20th October, 2012 were carried out to determine the residual levels of pyrethriods and the types used in the cultivation Accepted 16th November, 2012 of pepper (Capsicum annuum L.) at the Libga irrigation site in Ghana. Pepper samples were Published online 28th December, 2012 randomly taken from farmers in Libga to determine pyrethroids level using the US-EPA method 3510. Administration of questionnaires to 39 farmers was also carried out to know some spraying Key words: practices and types of pesticides used. Results indicated that Allethrin, Bifenthrin, Fenpropathrine, Pyrethroids, Lambda-cyhalothrin, Permethrin, Cyfluthrin, Cypermethrin, Fenvalerate and Deltamethrin residual Lambda-cynaiddinni, 1 ciniculini, Cypernennii, Cypernennii, 1 cypernenni, 1 cype Pyrethroid Type 1, Pyrethroid Type 2, Residual effects. 0.02 ± 0.00480 mg kg⁻¹, 0.1428 ± 0.1385 mg kg⁻¹ respectively. Fenvalerate that recorded a residual Capsicum annuum level equal to European Union's maximum residual levels. However, Allethrin, Bifenthrin, Lambdacyhalothrin, Permethrin, Cyfluthrin, Cypermethrin, and Deltamethrin had their mean residual levels below Eurpean Union's maximum residual levels. Fenpropathrine recorded a value that was above Eurpean Union's maximum residual levels. The implications of the above observations are that most pyrethroids insecticide used were not persistent in the cultivated pepper at Ligba and most pepper

sampled from Ligba have less potential health risk.

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INTRODUCTION

In Ghana vegetable production is a component of food security strategy (Parker et al., 2010). According to the World Health Organization (2003), pesticide residue levels will be high in fruits and vegetables that are mainly consumed raw or semi-processed compared to food groups of plant origin such as bread and others (Claeys et al., 2011). Pyrethroids is known to be another class of synthetic class of pesticides created in the late 1970's (Farnham, 2007) and reported to be the third largest class of pesticides (Wirtz et al., 2009). Pyrethroid pesticides have received phenomenal support by the agricultural community due to their very high activity, low use rates, broad spectrum of activity, excellent mammalian safety and cost-effectiveness (Watkinson, 1990). As a result it has been accepted in the market for pest control. There are two types of pyrethroids depending on their chemical structure and symptoms of exposure. Type I pyrethroids include allethrin, tetramethrin, resmethrin, d phenothrin, bioresmethrin, and permethrin. Type II pyrethroids in cypermethrin, cyfluthrin, deltamethrin, cyphenothrin, fenvalerate, and fluvalinate (Klaassen et al., 1996 and Ray, 1991). All foodstuffs intended for human or animal consumption in some part of the world

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are subject to a maximum residue level (MRL) of pesticides in their composition in order to protect animal and human health (IPC, 2012). A maximum residue level (MRL) is the highest level of a pesticide residue that is legally tolerated in or on food or feed. The amounts of residues found in food must be safe for consumers and must be as low as possible (European Commission, 2008). In Ghana pesticides are used to prevent or reduce crop loss by pests and diseases. For several decades, pesticides have been employed in agriculture not only to control and eradicate crop pests but also in the public health sector for disease vector control in Ghana (Hodgson, 2003).

Ghana is a large importer of pesticides (Herbicide, insecticide or fungicide) and importations have been on the increase since 2002 (Darko, 2009). With the large importation, the use of pesticide has increased over this period and is particularly elevated in the production of high-value cash crops and vegetables (Gerken *et al.*, 2001). Majority of the pesticides are employed in the forest areas or farming regions for the production of cereals, vegetables, legumes tubers and fruits in Ghana (Ntow, 2005; Amoah *et al.*, 2006) especially in the Ashanti, Brong Ahafo, Eastern and Western regions. Pepper (*Capsicum annuum* L) has always been part of Ghana's agriculture and diet. The crop requires sunny, semi-tropic or tropical conditions and annual rainfall of between 600 mm and 1,250 mm. Pepper has a relatively quick growing and harvesting period of 3 to 4 months in Ghana and is generally grown by local farmers during the rainy season (MiDA Ghana, 2010). Some important organic nutritive constituents of pepper include Vitamin C which prevents scurvy, and heals wound and also improves the immune system. Pepper also contains flavones which can result in the initiation of cancer allergies and heart disease (Craig and Beck, 1999). There has been a rapid rise in the quantity of pesticides used in agriculture over the past years (Hogson, 2003). Farmers tend to use large amount of pesticides during the entire period of growth of vegetables as well as during the fruiting stage. In addition, they ignore recommended waiting period between the harvest and last spray (Kumari et al., 2003). Owing to these practices, pesticides become inner part of vegetables which could be used by consumers thus creating health hazard (Kumari et al., 2003) since they are consumed directly without much processing or storage (Owago et al., 2009). Since the demand for vegetables is not seasonal, farmers attempt year round production wherever irrigation water is available (Amoah et al., 2006). Ligba in the Northern Region of Ghana is a community where irrigation water is available. In recent years, there has been serious concerns raised about health risk resulting from pesticide residues in food (Eskenazi et al., 2008). Some of these pesticides are known to be endocrine destructive or impair reproductive competence in male (Figa-Talamanca et al., 2001). The study was to assess the residual levels of pyrethroids pesticides in pepper (Capsicum annuum L.) by comparing with European Union maximum residual levels. The main objective of the study was to assess the types, uses and levels of pyrethroids pesticides residues in pepper (Capsicum annuum L.) at Libga.

MATERIALS AND METHODS

Study site

The study was conducted at Libga irrigation site which is in the Savalugu-Nanton district of the Northern Region of Ghana. The area under irrigation however is about 16ha. The major crops cultivated on site are rice, cowpea and pepper. The climate is the Guinea Savannah type (MoFA, 2011). The district receives an annual rainfall averaging 600mm and has an annual temperature of 34 $^{\circ}$ C. The District is generally flat with gentle undulating low relief (savelugunanton.ghnana districts.gov.gh) and located at longitude 9° 37′ 26.4″ N and latitude 0° 49′ 40.8″ W. The study consisted of both survey and experimental work.

SURVEY

Simple random sampling method was used in selecting thirtynine farmers farmers. Out of the thirty-nine farmers interviewed, nineteen were males and twenty were females. The survey was carried out to ascertain the type of pesticides used by farmers in Ligba in pepper cultivation, and to obtain some practices carried out by farmers when using pesticides and when pesticides are applied before harvesting.

Experimentation

Collection of Pepper Samples

The study started in February, 2012 by collecting six groups of pepper sample from the Ligba irrigation site. The irrigation

site was divided into two parts: upstream and down stream irrigation sites, based on the elevation of the site. Out of the six groups of pepper sampled, three groupd were collected from the upstream irrigation sites and labeled as UPS1, UPS2 and UPS3 and the other three from downstream irrigation site were labeled as DOS4, DOS5 and DOS6, respectively. Each sample group was collected from six different farmers using simple random sampling and was analysed for pyrethroid residue levels and compared to EUs maximum residual level.

Extraction of pyrethroids

The method of extraction used was the US-EPA 3510 that is mainly used for extracting multi-residue pesticides in non fatty crops as described by Keith (1996). The extraction was done with hexane as the solvent. Sodium hydrogen carbonate (NaHCO₃) was used to neutralize any acid that was present. The sample was weighed using Meller Toledo weighing balance and recorded as indicated in Table 1.

Table 1. Weight of pepper samples collected

UPSTREAM		DOWNS	DOWNSTREAM		
Farmer ID	Weight (g)	Farmer ID	Weight (g)		
UPS1	6.9	DOS4	6.8		
UPS2	7.0	DOS5	6.9		
UPS3	6.9	DOS6	6.9		

The weighed pepper sample was washed thoroughly with distilled water. The sample was placed in a blender and 5g of anhydrous sodium sulphate (Na₂SO₄) and 50 ml of hexane was added and grounded into a paste. The paste was transferred into a 25 ml conical flask with the help of a spatula. The blender was washed thoroughly with water and acetone before the next sample. It was repeated for the entire sample. Concentration was done to remove the solvent (hexane) using a rotary evaporator. Ethyl acetate (10 mL) was added and the mixture was shaken thoroughly. A 3g portion of aluminium oxide (Al₂O₃) and 1g of activated charcoal was added to the mixture followed by 1g of anhydrous sodium sulphate (Na₂SO₄) and the entire mixture was shaken vigorously for four to five hours. This was to ensure that enough of the pesticide residue dissolved in the ethyl acetate. The mixture was filtered into a 25ml labeled round bottom flask and then centrifuged at a speed of 1800 rpm for 5 min and the organic layer decanted into a 25ml conical flask.

Procedure for Clean-up

A 10 mm chromatographic column was filled with 3 g activated silica gel and topped up with 2-3 g of anhydrous sodium sulphate. Then, 5 mL of n-hexane was added to the column by allowing the tap to run. The residue in 2 mL n-hexane was then transferred onto the column and the extract rinsed three times with 2 mL hexane. The samples was sealed and placed in the refrigerator at the laboratory below room temperature to prevent evaporation of the ethyl acetate.

Gas Chromatography

The instrument that was used in the GC analysis is Varian CP-3800 GC. It is easily configured to perform online analysis for monitoring critical gas and liquid process streams. A 25 μ L glass Hamilton syringe was used to inject the GC samples. Only 1-2 μ L of the sample was injected onto the column. The syringe was examined carefully before it was filled. A small amount of the liquid was slowly drawn by raising the plunger and then pressed to expel the liquid back into the liquid. This served to "rinse" the syringe with the sample, ensuring that what was measured in the GC run was the composition of the mixture. The rinsing process was repeated twice. Then the plunger was slowly drawn up again while the needle was in the liquid and the syringe was carefully filled with the liquid. Small air bubbles in the syringe did not affect the GC run. The sample was injected into the injector port. Two things were done sequentially and quickly. The needle of the syringe was pushed through the injector pot and immediately the plunger was pressed to inject the sample. Then immediately the start button on the recorder was pressed. A bit of resistance was felt from the rubber septum in the injector port. The recorder was observed for some time. Within several minutes, it recorded several peaks and the GC run was ended. The recorder printed out the peaks, the retention times and the areas under the peaks. The pesticide residue concentration was then deduced from the following equation:

$$\label{eq:residue} \begin{split} \text{Residue level} = \underline{\text{concentration in final extract} \times \text{final volume of extract}} \\ & \text{Weight of sample} \end{split}$$

RESULTS

Survey

Pesticides use and practices by Farmers

Results in Table 2 indicate the various types of pesticides used to control insect pests in pepper production at the study community. From the survey conducted a total of 12 different types of pesticides were identified.

 Table 2. Pesticides used by farmers to control pest in pepper in

 Libga

Pesticides(Trade names)	Active ingredient
Dursban 3.5EC	Chlopyriphos
Thiodan	Endosulphan
Kombat 2.5EC	Lamda cyhalothrine
Polythhrine	Cypermetrine
PAWA 2.5EC	Lamda cyhalothrine
Sunphosate	Deltamethrin
Rambo 2.5EC	Deltamethrin
Lamda 2.5EC	Lamda cyhalothrine
Cocostar	Bifenthrine+pirimiphasmethyl
Attack	Emamectin benzoate
Rimon 10EC	Noraluran.
Actellic	Pyrimiplus methyl

Response of farmers to brands of pesticides used on the farm

According to the field survey, 21 farmers representing 54% change the brand of pesticides during the cultivation of pepper, and 18 representing 46% use the same brand of pesticides throughout cultivation of pepper.

Farmers' practices

Mixing of different brands of pesticides before application

At Ligba irrigation site, some practises of the farmers included mixing of two or more pesticides (pesticide cocktail) for the control of insect pest on their respective farms. Out of the total farmers interviewed, twenty eight farmers used pesticide cocktail in controlling pest and eleven farmers did not practice cocktail formulation.

Reasons for cocktail formulation

Out of the twenty eight farmers who practiced cocktail formulation, 25% of this group mixed pesticide because they are uncertain about the effectiveness of pesticides, 39.3% also mix pesticides because they are not sure about the quality of pesticides, with ten farmers (35.7%) mixing pesticides because other farmers practised that.

How long the farmers wait after spraying before harvesting

It was also revealed that out of the thirty nine respondents, seventeen farmers (43.6%) usually wait a week after pesticide application before harvesting their pepper where as 15.4% and 7.7% respectively wait for a day and three (3) days before harvesting. Thirteen farmers (33.3%) had no particular time in applying pesticides to their pepper.

Time of the day farmers apply pesticide

It was also revealed that out of the total farmers interviewed 19 representing 48.6% sprayed in the morning (6am-11am), 18 representing 45.7% sprayed in the evening (4-7pm) and only 2 representing 5.7% (12- 3pm) sprayed in the afternoon.

Pesticide residue

Concentrations of Pyrethroids Pesticides Residues inPepper

The residual levels of the various groups of pyrethroids identified in pepper from Ligba was compared with EUs maximum level (EU regulation (EC) No. 396/2005). Pyrethroids pesticide residues detected from the pepper sampled included Allethrin, Bifenthrin, Cyfluthrin, Cypermethrin, Deltamethrin, Fenpropathrin, Fenvalerate, Lambda-cyhalothrin and Permethrin. Allethrin and Bifenthrin were not detected in sample DOS1 and DOS3, respectively. Cypermethrin was only detected in sample UPS1 and Lambda-cyhalothrin was not present in DOS1 sample as shown in Table 3. Deltamethrin had the highest maximum residual level of 0.124 mg kg⁻¹. Whilst the lowest pyrethroid residue was Allethrin (0.0015 mg kg⁻¹).

Table 4 shows pyrethroid residual levels in pepper samples from up stream and down stream. Analysis of variance revealed that there was no significant difference between pesticide residual level of Allethrin in pepper from up stream and down. Similar observation were made for Bifenthrin, Cyfluthrin, Cypermethrin, Lambda-cyhal and Permethrin. These pesticide residues in the pepper were below EUs MRLs as shown in Table 4. Although there was on significant difference between Fenpropathrin concentration in sample from upstream and downstream, the mean residual level of Fenpropathrin (0.0250 mg kg⁻¹) at upstream was above the EUs MRLs by a factor of 2.5. However there was significant difference between Fenvalerate residual concentration at up stream and down stream. A similar observation was made for Deltamethrin. Fenvalerate and Deltamethrin both recorded high residual concentration of 0.0241 and 0.257 mg kg⁻¹ respectively in samples from up stream and were greater than EUs MRLs of 0.2 and 0.01 mg kg⁻¹ respectively, with low residual concentration in samples from downstream as shown in table four below.

Table 3. Pyrethroids pesticide residue from upstream and down stream samples

			М	g kg ⁻¹				
Pyrethroid residues	UPS1	UPS2	UPS3	DOS1	DOS2	DOS3	Minimum	Maximum
Allethrin	0.0027	0.0024	0.0045	ND	0.003	0.0015	0.0015	0.0045
Bifenthrin	0.0124	0.0121	0.0082	0.009	0.0068	ND	0.0068	0.0124
Cyfluthrin	0.0472	0.0408	0.0327	0.0245	0.0316	0.0474	0.0474	0.0245
Cypermethrin	0.0088	ND	ND	ND	ND	ND		0.0088
Fenvalerate	0.0237	0.0251	0.0236	0.017	0.0166	0.0135	0.0135	0.0251
Deltamethrin	0.3201	0.1586	0.2915	0.075	0.0075	0.0041	0.0041	0.3201
Fenpropathrin	0.0143	0.0532	0.0076	0.0196	0.0134	0.0135	0.0076	0.0532
Lambda-cyhalothrin	0.0067	0.0069	0.0109	ND	0.0069	0.0075	0.0067	0.0109
Permethrin	0.0108	0.0098	0.0166	0.0234	0.0106	0.0062	0.0062	0.0234

UPS1, UPS2, and UPS3 mean pepper samples from farmers at up stream, DOS1, DOS2, and DOS3 mean sample from farmers at down sream; ND means Not detected

Table 4. Means of pyrethroids residual level at upstream and downstream

	mg kg ⁻¹					
Pesticide residue	UPS Means	DOS means	F.Pr	Mean Standard Deviation	MRLs	
Allethrin	0.0032	0.0015	0.193	$0.0023^{*} \pm 0.00109$	0.01	
Bifenthrin	0.0109	0.0053	0.136	$0.0081^* \pm 0.00453$	0.2	
Cyfluthrin	0.0402	0.0345	0.511	$0.0374^{*} \pm 0.00927$	0.3	
Cypermethrin	0.0029	0.0000	0.374	$0.0015^* \pm 0.00359$	0.05	
Fenvalerate	0.0241	0.0157	0.002	$0.02^{***} \pm 0.00480$	0.02	
Deltamethrin	0.257	0.0290	0.014	$0.1428^{*}\pm0.1385$	0.2	
Fenpropathrin	0.0250	0.0155	0.543	$0.0203^{**} \pm 0.01658$	0.01	
Lambda-cyhalothrin	0.0082	0.0048	0.291	$0.0065^* \pm 0.00354$	0.1	
Permethrin	0.0124	0.0134	0.866	$0.0129^{*} \pm 0.00613$	0.05	

UPS means upsreams, DOS meansDown Stream, F.Pr means Fisher Probability, MRLs means Maximum Residual Limits according EU Regulation (EC) No. 396/2005 . * means pesticide residual level is below EUs MRLs. ** means mean pesticide residual level is above EU MRLs and *** means mean pesticide residual level is equal to EU MRLs

In general, the present study showed the following pyrethroid residues; Allethrin, Befinthrin, Cyfluthrin, Cypermethrin, Deltamethrin, Fenvalerate, Fenpropathrine, Lambdacyhalothrin and Permethrin which were present in the sampled pepper, but there was no significant differences between the pyrethriod residues identified.

DISCUSSION

Farmers and pesticide usage

The pyrethroid residues identified indicated that farmers in Ligba used pesticides that had pyrethriods as an active ingredient. In addition, seven pesticides had pyrethroids as their active ingredient out of the twelve pesticides used by the farmers in pesticide control. This was an indication that farmers in Ligba are gradually accepting the use of pyrethroid pesticide. This agrees with Bempah et al., (2011), who identified more of pyrethroid pesticide residues in fruits and vegetables in the Kumasi metropolis than organochlorine pesticide residues. Based on the pyrethroids identified, pesticides with allethrin, cyfluthrin, fenvalerate, fenpropathrin and permethrin as active ingredient were also used by farmers at Ligba irrigation site in controlling pest. The malpractices noticed in Ligba such as pesticide cocktail formulation and the fact that some farmers sprayed pesticide on their farm a day or three days before harvest was due to inadequate knowledge of pesticide usage, as well as poor communication between farmers and extension officers.

Pyrethroid's residual levels

The detection of Allenthrin, Bifenthrin, Fenpropathrine, Lambda cyhalothrin, Cyfluthrin, Cypermethrin Deltamethrin and Fenvalerate in the pepper sample were similar to reports made by Armah (2011) who identified nine pyrethroids residues in cabbage at Cape Coast in Ghana with the exception of Fenpropathrine. The low concentration of Allenthrin $(0.0023 \text{ mg kg}^{-1})$, Bifenthrin $(0.0081 \text{ mg kg}^{-1})$, Cyfluthrin $(0.0374 \text{ mg kg}^{-1})$, Cypermethrin $(0.0015 \text{ mg kg}^{-1})$, Lambda cyhalothrin (0.0065 mg kg⁻¹), and Permethrin (0.0129 mg kg⁻¹) residue in pepper compared to EUs maximum residual level could be attributed to the time of pesticide application before harvest and also as a result of late harvest. Late harvest would allow the active ingredients of the pesticides to be degraded by the pepper plant. According to Health Canada (2007) the preharvest interval (PHI) is a function of a pesticide's use pattern and of the amount of pesticide residues allowed on the crop at harvest. Residual levels of a crop are affected by the crop's growth, environmental conditions such as rain or UV radiation and the microorganisms living in the plants and in the soil. The PHI must therefore be long enough to allow for the pesticide residues in the harvested crop to degrade to a level that is acceptable.

In addition, farmers using this group of pesticides were following strictly according to users guide, hence preventing the misuse of the pesticides. The grand mean concentration of Deltamethrin (0.1428 mg kg⁻¹) was below EUs MRLs (0.2 mg kg⁻¹). The concentration of Deltamethrin $(0.257 \text{ mg kg}^{-1})$ in pepper sample from upstream was above EUs MRLs (0.2 mg kg⁻¹). The high concentration of deltamethrin in the pepper samples could have resulted from a short pre-harvest interval. the pesticide's active ingredient Due to the short PHI deltamethrin was not degraded before harvest. Also, farmers activities such as pesticide cocktail formulation can contribute to the high concentration of deltamethrin. Since two different trade names of a pesticide can have the same active ingredient. According to Frank and Kellner (2000) laboratory animal studies have demonstrated toxicity in response to acute exposure to technical grade deltamethrin, as well as, to acute exposure to product formulations containing deltamethrin as the active ingredient. The primary toxic signs were characteristic of agents that disrupt the autonomic nervous system and many of these signs were consistent with the neurotoxicity associated with pyrethroids. These signs included excessive salivation, decreased activity, labored breathing, gasping, impaired limb function, ataxia, loss of righting reflex, tremors, convulsions, and lethality. Furthermore, signs of autonomic nervous system dysfunction (e.g., liquid feces, vomiting, and tremors) have been reported in studies designed to examine the effects of multiple exposures to deltamethrin. These health consequences are possible to humans when exposed or ingest products with high deltamethrin residues. Fenpropathrine mean residual level (0.0203mgkg⁻¹) was above EUs MRLs of 0.01 mg kg⁻¹ that is twice the concentration of EUs MRLs value. This resulted from factors considered in the high concentration of deltamethrin. In addition, the farm land on which the pepper was cultivated could have had high levels of the compounds (pyrethroids) as part of it composition. In high concentration of fenpropathrin when ingested by a laboratory animal, symptoms of poisoning include muscle incoordination, tremors, convulsions, nerve damage and weight loss. Fenpropathrin is suspected to be an endocrine disruptor (Kegley et al., 2010).

Experimental studies with this pesticide have demonstrated toxic activity in laboratory animals, primarily related to neurotoxicity and this is presumable for humans as well. The factors influencing high concentration of deltamethrin as well as fenpropathrin can be attributed to the equal level of fenvalerate residual level (0.02 mg kg⁻¹) to EUs MRLs (0.02 mg kg⁻¹). Although fenvalerate is considered to have low acute toxicity to mammals, it is having severe neurotoxic effects and estrogenic activity causing endocrine disorders. Hence fenvalerate is enlisted as one of the neuroendocrine disrupting chemicals (EDCs) and has aroused worldwide concern (Junhe et al., 2006). At sub-lethal doses in rodents, fenvalerate produces neurological toxicity but no histological damage; at higher doses, pathological alterations in peripheral nerves occur (Bradbury and Coats 1989). Rats given acutely toxic doses of fenvalerate showed histopathological changes such as axonal swelling and degeneration and myelin fragmentation of the sciatic nerve; the significance of these findings is unclear (Gray and Soderlund 1985). Cypermethrin was detected in only one sample out of the six samples. This indicates that pesticide with cypermethrin as an active ingredient were not used by most farmers at Libga irrigation site. It might have also resulted from degradation of cypermethrin by the pepper plant before harvested for pyrethroid detection or analysis.

Conclusion

Most of the pyrethroids residues detected were below EUs MRLs, and this indicated that farmers at Libga irrigation site applied pyrethroid pesticides in the appropriate manner. In addition, this confirms that pesticides with pyrethroids as active ingredient have rapid rate of degradation in the environment as well as in plant unless misapplied as in the case of Fenpropathrin and Deltamethrin. Further studies on pyrethroids residues using other vegetables would provide an in depth knowledge about pyrethroids residues in vegetables

produced in Ghana and the use of pesticides. This could assist in policy making on what pesticide should be accepted and used in other to achieve food safety and security.

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