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Analysis of Deltamethrin Residue Amounts Using HPLC in Some Vegetables Consumed in a Rural Area - Makuyu, Kenya

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Abstract:
Vegetables are brimming with fibre, plus a whole range of vitamins and minerals, and because they are low in calories, they make an important and healthy addition to any diet. Scientific studies have shown that people who eat a lot of vegetables may have a lower risk of getting illnesses, such as heart disease and some cancers. For this reason, health authorities recommend that you eat at least five portions of fruit and vegetables every day. Pests, diseases, and weeds destroy vegetables reducing their yield and causing blemish thus making them unfit for sale and consumption. Pesticides maintain the high levels of vegetable production in Kenya. There are many pesticides in use today including; insecticides, acaricides, nematocides, herbicides, and avicides. Pyrethroids are the most commonly used insecticides. The pyrethroids in use include; deltamethrin, lambda-cyhalothrin and chismethrin. However, although vegetables are widely consumed by almost everybody in Kenya, there is little work reported on the analysis of pesticides residues in vegetables. Most of the vegetables are grown in the rural areas, with most farmers growing them on small-scale and hence these vegetables never the reach cities. In this study deltamethrin was analysed in selected vegetables which include; kales, cabbages and tomatoes grown I rural area during the dry and wet seasons. The vegetables were bought from different sellers in Makuyu market. They were then homogenized to give analytical samples. Deltamethrin residues were extracted from the samples using organic solvents. The residues were then determined using high performance liquid chromatography (HPLC). Analyses of data was done using ANOVA, t-test and regression analysis. The deltamethrin mean residue levels ranged between 0.0800±0.1300 and 0.1400±0.0800 mg/kg during the dry season and between 0.0012±0.0016 and 0.1100±0.0080 mg/kg during the wet season. The study showed that deltamethrin mean residue levels were higher during the dry than during the wet season in the vegetables analysed. The differences were not statistically different for most of them. During the dry season the deltamethrin mean residue levels in the vegetable samples were all higher than the Acceptable Daily Intake (ADI) of 0.02 mg/kg, but they were all lower than the Maximum Residue Levels (MRLs) of 0.2 mg/kg. During the wet season the deltamethrin mean residue levels were lower than the ADI except in kale samples. During the same wet season the deltamethrin mean residue levels were all lower than the MRLs.

Keywords : *Vegetables, rural area, pyrethroids, deltamethrin residues and HPLC*

1. Introduction

Vegetable is a culinary term, which generally refers to an edible part of a plant. All parts of herbaceous plants eaten as food by humans, whole or in part, are normally considered as vegetables (Swedenborg, 2003)[1]. Thus potatoes, bean, onions, cabbages and tomatoes are vegetables. Vegetables provide vitamins, minerals and flavonoids (Gallow *et al.*, 1993)[2]. These substances contained in vegetables are very important, for instance vitamin C is required for the production of collagen - the substance that gives structure to muscles, veins, arteries, bones and cartilage. A study by Unlu *et al.* (2005)[3] reported that avocados act as a nutrient "booster," allowing the body to significantly absorb more nutrients like alpha-carotene, beta-carotene and lycopene found in fruits and vegetables. Vegetables in the daily diet have been strongly associated with reduced risk for some forms of cancer, heart disease, stroke, and other chronic diseases (Goldberg, 2003)[4]. Vegetable production is one of the major branches of Horticulture. They are considered as an asset for providing a good source of income to the growers and they form a vital part of the human diet (Gallow *et al.*, 1993)[2]. Vegetables are also considered a cheaper source of natural supplementary food because they contain essential nutrients. Many of the vegetable crops posses' high medicinal value in curing certain diseases. For instance, onion and garlic have been reported to posses' anti-bacterial property, and to lower the rising blood sugar of rabbits (Shan, 1989)[5]

Unfortunately, pest, diseases, unfavorable conditions, and weeds can attack vegetables and reduce them in terms of quality and quantity. As a result, commercial vegetable growers spend a great deal of money on pesticides so that they get the highest possible yields and completely unblemished produce for marketing. There are now many chemicals with which pests, diseases and weeds can be controlled (Shan, 1989)[5]. Almost all these chemicals are poisonous to creatures besides those, which they are intended to kill. Thus, they can be very dangerous to the life of humans, animals, fish, and birds. The class of pesticides commonly used on vegetables is the insecticides, and mostly the pyrethroids insecticides such as deltamethrin (Shan, 1989)[5]. The effect of pesticides on the environment depends on several factors such as climate, in particular temperature and rainfall; soil type and nature of the vegetative cover; biotic activity; light intensity; agricultural practices; and mode of introduction of the pesticide into a particular environmental compartment (Mark, 2003)[6]. There is therefore, need for monitoring the levels of pesticide residues in the vegetables.

The main aim of this study therefore was to determine the levels of deltamethrin residues in some vegetables. Deltamethrin is a pyrethroid insecticide that kills insects on contact. Trade names for products containing deltamethrin include decis, butoflin, butoss, butox, cislin, crackdown, cresus, decis-prime, K-othrin, and K-otek (Gammon *et al*., 1981)[7]. It is an insecticide used in cotton, vegetables, cereals, ornaments and field crop (Bradbury and Coats, 1989)[8]. It is used to control apple and pear suckers, plum fruits moth, caterpillars on brassicas pea and aphids on vegetables (Bradbury and Coats, 1989)[8]. The molecular formula is $C_{22}H_{19}Br_2NO_3$. Deltamethrin has very broad-spectrum control and is considered the most powerful of the synthetic pyrethroids adding up to three orders more active than some pyrethroids (Bradbury and Coats, 1989)[8]. Formulation includes emulsification concentrate, flowable formulations, and granules. Deltamethrin can induce skin sensations in people working with the compound (FAO/WHO, 1986)[9]. Several non-fatal cases of poisoning have been reported through occupational exposure resulting from neglect (FAO/WHO, 1986)[9]. These include numbness, itching, tingling, and burning of the skin; occasionally, a transient popular or blotchy erythema has been described and most of these symptoms are transient and disappear within 5-7 days (FAO/WHO, 1986)[9]. In a non-aqueous vehicle, the acute oral toxicity of deltamethrin is high to moderate with LD_{50} values of 19-34 mg/kg (rat) (Mestres *et al*., 1986)[10]. Clinical signs of poisoning by deltamethrin include tremor, salivation, and convulsion (Mestres *et al*., 1986)[10].

2. Experimental and Methodology

2.1. Sampling and Sample Pre-Treatment

The vegetable samples analysed in this study were kales (sukumawiki) (*Brassica oleracea C.),* cabbage (*Brassica oleracea A.)* and tomatoes (*Lycopersicon osculentum*), which are the most common vegetables in the Kenyan market. Plates 2.0 and 2.1 show researcher buying cabbages for the study and Kales growing in a garden respectively. The samples were bought randomly from different sellers in Makuyu market in Kenya during the dry season and wet season. The vegetables were then sorted out, weighed to some weight and then homogenized to make a representative sample. The samples were finally put in clean polythene bags, labeled and then stored safely in a refrigerator at –4ºC awaiting extraction and analysis. A total of five laboratory samples for each vegetable type were obtained. Each sample was run in duplicate.

Figure 1: Researcher buying cabbages for the study

Figure 2: Kales growing in a garden

2.2. Cleaning of Glass and Plastic Containers

Glassware are likely to absorb substances on their walls. It is thus important to clean them well before using them for any analytical work. The glassware used in this study were cleaned by soaking them for 12 hours in freshly prepared chromic acid. They were then rinsed with distilled-deionised water. They were then soaked in distilled-deionised water for about 6 hours to leach off any adsorbed chromic ions. Finally they were dried in an open rack after rinsing them with fresh distilled-deionised water. Plastic containers were thoroughly cleaned with detergents and then rinsed several times with 6 M Analar nitric acid. They were then rinsed thoroughly with distilled-deionised water and dried in an open rack. They were then kept under lock and key until required.

2.3. Reagents and Solvents

All the chemicals and reagents used in this research were of analytical grade and included;

- Hexane-Glass distilled–from Kobian distributors limited (Nairobi, Kenya)
- Acetone- Glass distilled–from Kobian distributors limited (Nairobi, Kenya)
- Florisil–from Kobian distributors limited (Nairobi, Kenya)
- Diethyl ether- Glass distilled–from Kobian distributors limited (Nairobi, Kenya)
- Analytical standard for deltamethrin-from Britain at Aldrich limited (Britain)
- Analytical standard for lambda-cyhalothrin-from Britain at Aldrich limited (Britain)
- Acetonitrile- HPLC grade–from Kobian distributors limited (Nairobi, Kenya)
- Water –Distilled and deionised HPLC grade

2.4. Preparation of Standards

The standard used in this study was deltamethrin. A 0.25 g portion of deltamethrin standard (equal to the weight of the sample used) was weighed into a 250 mL volumetric flask and dissolved to the mark with methanol to make a solution of 0.001 g/mL. The preparation of the standards followed the same procedure used to prepare the samples as already explained. This means that the known concentration of 0.001 mg/g of deltamethrin standard was used in the calculations in order to determine the unknown residue concentrations of the samples in mg/kg. Standards were stored at 4 ^{o}C.

2.5. Preparation of Samples

The weighed and stored samples were rinsed in organic free water and then blended in a mixture of solvents (Hexane and acetone in ratio of 1:1) (Sapiets *et al*., 1984)[11] with a blender for 5 minutes. They were then kept refrigerated for 16 hours for proper extraction to take place. Filtration with Whatman filter paper No.1 was done followed by concentration using a rotatory vacuum evaporator (Rotavapor R with a bath temperature of 40 ^{o}C), to remove much of the acetone.

The pre-concentrated sample was then cleaned-up using a chromatographic column packed with florisil and a mobile phase composed of a mixture of diethyl ether and hexane in the ratio of 1:6 (Sapiets *et al*., 1984)[11]. The cleaned-up sample was then concentrated again to remove the solvents. In this case, the standards having a known concentration enabled us to determine the unknown concentrations of the sample.

2.6. Analysis of Samples for Deltamethrin Using HPLC-DAD

A 0.25 g portion of the cleaned up sample was weighed into 250 mL volumetric flask, then dissolved to the mark with methanol. Equal volumes of samples and standards were prepared using methanol with a known concentration of the standard. A 20 μL portion of the sample and standard solutions was injected into the HPLC. The concentration of the standard and peak area of each peak enabled us to calculate the concentration of the residues. The HPLC elution used in this study was the isocratic elution, and was carried out at International Center of Insects Pathology and Ecology (ICIPE). The HPLC-model Beckman system gold series coupled with diode array detector module 168, with variable injector and wavelength detector was used. Detection for deltamethrin was fixed at 290 nm. Separation was carried out on reverse phase octyl column (25cm × 4.6 mm) containing 0.5- μm particle size from sigma Aldrich Germany, maintained at ambient temperature.

2.6.1. The HPLC was Operated Under the Following Conditions

- Eluent;
  - Acetonitrile 90%
  - Water 10%
- Elution: Isocratic
- Flow rate: 1.4 mL/min
- Injector : Injection volume- 20 μL

2.7. Analysis of Data

The data was evaluated using mean, the standard deviation, t-test and one-way ANOVA. The significant tests were done at p= 0.05 (Miller and Miller, 1992)[12].

3. Results and Discussions

Deltamethrin residue levels in kales, cabbages and tomatoes obtained from Makuyu were determined using HPLC-DAD, after a column clean-up with florisil. The raw data for the results obtained are as shown in Tables 1 and 2

Samples	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**
Kales	ND	0.1300±0.0000	0.1300±0.0140	0.1600±0.0140	0.1700±0.0070
Cabbages	0.2000±0.0000	0.0400±0.0000	0.2000±0.0100	0.0600±0.0010	0.2000±0.0140
Tomatoes	0.3000±0.0100	0.0500±0.0014	0.0400±0.0000	ND	ND

Table 1 Raw data of deltamethrin residue levels in mg/kg of duplicate vegetable samples from Makuyu market during the dry season n=2

Key:
S_1-S_5- Sample 1- sample 5
ND- Not Detected

The result in Table 2shows that most of the vegetable samples that were analysed during the dry season had deltamethrin residue levels with tomatoes having the highest levels.

Samples	**S_1**	**S_2**	**S_3**	**S_4**	**S_5**
Kales	0.0800±0.0050	0.0300±0.0014	0.2000±0.0050	0.0400±0.0014	0.2000±0.0140
Cabbages	0.0030±0.0003	ND	0.0030±0.0001	ND	ND
Tomatoes	0.0175±0.0001	ND	0.0016±0.0000	0.0020±0.0000	ND

Table 2: Raw data of deltamethrin residue levels in mg/kg of duplicate vegetable samples from Makuyu market during the wet season

Key:
S_1-S_5- Sample 1- sample 5
ND- Not Detected

Deltamethrin levels in kales obtained in dry season ranged from 0.1300 to 0.1700 mg/kg, while for cabbages the levels ranged from 0.0400 to 0.2000 mg/kg and for tomatoes deltamethrin levels ranged from non-detectable to 0.3000 mg/kg.

It can be seen from Table 2 that deltamethrin residue levels were very low in most of the vegetable samples analysed while in others nothing was detected during the wet season. All the kale samples analysed during the wet season had deltamethrin residues ranging from 0.0300 to 0.2000 mg/kg, while in cabbage and tomatoes only two samples had detectable levels of deltamethrin during the wet season.

The mean residue levels of the results in Tables 1 and 2 are as shown in Table 3

Vegetables	**Dry season**	**Wet season**	**$t_{(8, 0.05)}$**	**$t_{calculated}$**	**Difference**
Kales	0.1200±0.0300	0.1100±0.0080	2.31	0.71	Not significant
Cabbage	0.1400±0.0800	0.0012±0.0016	2.31	3.87	Significant
Tomatoes	0.0800±0.1300	0.0042±0.0075	2.31	1.30	Not significant

Table 3: Mean residue levels (Mean ± SD, n=10) of deltamethrin in mg/kg units in the samples obtained from Makuyu markets during the dry and wet seasons

From Table 3 the mean residue levels of deltamethrin in the vegetable samples obtained from Makuyu market, ranged from 0.0800 to 0.1400 mg/kg during the dry season, with cabbage having the highest mean residue levels and tomatoes the lowest, and from 0.0012 to 0.1100 mg/kg during the wet season with kales having the highest levels and the cabbage samples with the least. The mean residue levels of deltamethrin in the vegetable samples analysed during the dry season were generally higher than those analysed during the wet season although the difference was not statistically significance for most of them. The graphical presentation of this phenomenon is shown in Figure 3.0. Lee and Seeneevassen (1997)[13] reported a deltamethrin residue levels in cabbage of 0.0170 mg/kg, which were much lower than the deltamethrin residue levels obtained in the cabbage samples analysed from the Makuyu market during the dry season with a mean of 0.1400 mg/kg but was higher than the residues obtained during the wet season of 0.0012 mg/kg.

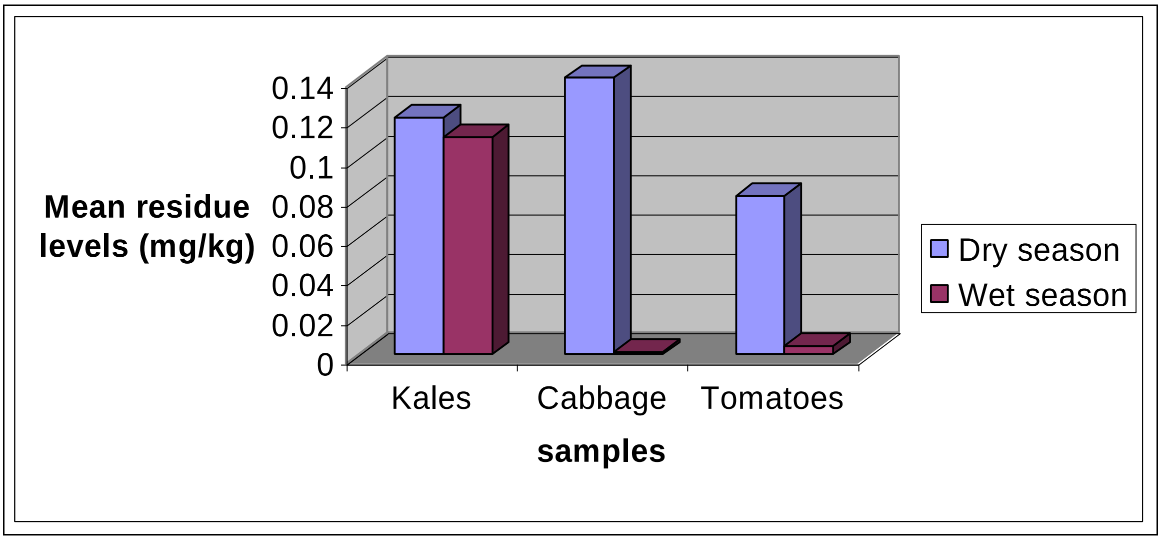

Figure 1: A graph showing the mean residues levels of deltamethrin in vegetable samples analysed from Makuyu markets

As can be seen from Table 3.0 and Figure 1, during the dry season, the deltamethrin mean residue levels in kales, cabbages and tomatoes which were 0.0800, 0.1200 and 0.1400 mg/kg respectively, were all higher than those obtained during the wet season although for most of them the differences were not statistically significant. This therefore, shows that during the dry season consumers were exposed to higher levels of deltamethrin residues levels from vegetables than during the wet season. This could probably be as a result of most farmers not applying pesticides on vegetables during the wet season. This is because during the wet season the aphids and other insects do not attack the vegetables as such. The reproduction rate of these insects is also very low during the wet season as most of their eggs and larvae are destroyed by the rains and weather. Also during the wet season there is wash off of pesticides from the vegetables by the rainwater. Another reason that may have contributed to this is that during the wet season is that the humidity tends to be high which favors the degradation of the pyrethroids. The eggs normally hatch during the dry season due to the favorable dry weather and hence increasing the number of insects and pests, which probably makes the farmers to apply a lot of pesticides on the vegetables in order to eradicate them. The results from this study indicate that deltamethrin mean residue levels in the samples analysed from Makuyu market were higher during the dry seasons than during the wet seasons although the differences were not significant. During the dry season the deltamethrin mean residue levels in the vegetable samples were all higher than the Acceptable daily intake (ADI), which is, 0.02 mg/kg by FAO and WHO (FAO/WHO, 1996)[14], while all of them were lower than the Maximum residue levels (MRLs) which is 0.2 mg/kg by FAO/WHO (FAO/WHO, 1996)[14]. During the wet season the deltamethrin mean residue levels in kale samples were higher than the ADI but lower than the MRLs while in cabbage and tomato samples the residues were lower than both the ADI and MRLs.

4. Conclusion

The current study indicates that most of the vegetable samples analysed during the dry and wet seasons had deltamethrin residues. The results also show that most of the vegetable samples studied had higher levels of deltamethrin than the ADI during the dry season but during the wet season the levels were in the safe range except in kales. Therefore the farmers should wait for the recommended elapse time before harvesting the vegetables and also they should apply the right amount of the pesticides to the vegetables as is recommended by the manufacturers. The consumers on the other hand should make sure they do wash and rinse their vegetables thoroughly with flowing or enough water before consumption.

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6. References

1. Swedenborg, E., (2003). Swedenborg concordance 1888. Kessinger publishing. Pp 502. Sweden.
2. Garrow, J. S., and James, W. P., (1993). Human Nutrition and Dietetics 9th Edition, Churchill Livingstone, Melbourne.

3. Unlu, N. Z., Bohn, T. Clinton, S. K., and Schwartz, S. J. (2005). Carotenoid absorption from salad and salsa by humans is enhanced by the addition of avocado or avocado oil. Journal of Nutrition, 135: 431-436.
4. Goldberg, G. (ed). (2003). Plants: Diet and Health. The report of a British Nutrition Foundation Task Force. Blackwell Science, Oxford U.K., 347 pp.
5. Shan, M. K.G., (1989). Production technology of vegetable crop. Academic press, New York. pp 27-38.
6. Mark, F. Z., (2003). Pesticide use in Zimbabwe. (Impact on Lake Kariba, a tropical freshwater ecosystem). IN: Pesticides Residues in Coastal Tropical Ecosystems. Published by Taylor and Francis, 11 New Fetter Lane, London EC4P 4EE.
7. Gammon, D.W., Brown, M. A., and Casida, J. E., (1981). Two classes of pyrethroid action in the cockroach. Pestic. Biochem. Physiol. 15: 181-191.
8. Bradbury, S.P., and Coats, J.R., (1989). Toxicokinetics and toxicodynamics of pyrethroid insecticides in fish. Envron. Chem. 8: 373-380.
9. FAO/WHO., (1986). Cyhalothrin: In: 1986 Evaluations of some pesticide residues in food, Part 1: Residues, Rome, Food and Agriculture Organisation of the United Nations. pp 78.
10. Mestres, R., Atmawijaya, S., And Chevallier, C., (1986). Methods for the study and determination of pesticide residues in cereal products. XXXIV. 1. Organochlorine, organophosphorous, pyrethrin, and pyrethroid pesticides. Ann. Falsif. Expert. Chim. 72: 577-589.
11. Sapiets, A., Swaine, H., and Tandy, M. J., (1985). Analytical methods for pesticides and plant growth regulators. Academic press, New York. pp 33.
12. Miller, J C. and Miller, J. M. (1992). Statistics for Analytical Chemistry. Ellis Horwood West Sussex England.
13. Lee, F.C., and Seeneevassen, S., (1997). Monitoring of some insecticides residues in vegetables and fruits at the market level. Ministry of Agriculture, Fisheries and Cooperatives. (Unpublished report).
14. FAO/WHO., (1996). Pesticide residues in food – Maximum residue limits. 2B. Second Edition. 1996.Codex Alimentarius.