Determination of Maximum Residue Levels (Mrls) Of Lambda-Cyhalothrin (Karate 1.75 Ec) and Pre-Harvest Interval (Phi) on Tea in Kenya

Emily C. Cheshari[1] Vincent Sudoi[2] Ward J. Mavura[3] John K. Wanyoko[4]
[1] Department of Chemistry and Biochemistry, Laikipia University, Box 1100 -20300, Nyahururu
[2] Department of Environmental Biology, University of Eldoret, Box 1125-30200 Eldoret.
[3] Department of Chemistry, Jomo Kenyatta University of Agriculture and Technology, Box 62000-00200 Nairobi
[4] Department of Biochemistry, University of Eldoret, Box 1125-30200 Eldoret

Abstract

Globally, 1031 species of arthropods are associated with the intensively managed tea. All parts of the plant, leaf, stem, root, flower, and seed, are fed upon by at least one pest species, resulting in an 11%–55% loss in yield if left unchecked, hence the need for use of pesticides. With climate change and increasing temperatures the need is greater. Lambda-cyhalothrin is an insecticide currently registered and used in Kenya for control of several insect and mite pests in diverse crops. This study established residue levels for lambda-cyhalothrin in fresh tea leaves, black tea and brewed tea. The study evaluated the effect of tea preparation procedures on pesticide residue levels in tea and monitored the decline of pesticide residues under normal harvest time intervals. The samples were collected at various intervals after application of the pesticide at maximum proposed application rate of 3.0 Lha^{-1} (i.e. worst-case conditions allowable) according to instructions on the label. The study was carried out at Timbilil estate of Tea Research Foundation of Kenya in Kericho. Extraction of lambda-cyhalothrin from Karate treated samples was accomplished using 50% acetone in hexane. Analysis of the samples was done by Gas Chromatography (GC). The pesticide residue concentrations in the tea samples were calculated using the power curve fit; $y = bx^m$. Results show that the levels of the pesticide residues decrease with increase in the pre-harvest interval days. The processing and brewing of tea appear to affect the residues of lambda-cyhalothrin most significantly. The residue levels from the study were lower than the maximum residue levels (MRLs) allowed within the European Union. Therefore, if this pesticide is used according to the established pattern it will pose no risk to the consumers of tea.

Key Words: Residue levels, lambda-cyhalothrin, PHI, MRLs Tea, Kenya

INTRODUCTION

Pesticides have continued to be of interest to toxicologists, biologists, ecologists, agriculturalists and analytical chemists due to their inherent toxicity. Analytical chemists have devoted time to research the specific area of analytical chemistry of pesticides (Moye 1981). With development of chlorinated hydrocarbons and their widespread use in agriculture it became apparent that residues in food were important and thus the study of pesticides in food is a major component in pesticide development. Each crop or food product for which a pesticide is registered must be analyzed for residues and a tolerance established (McEwen and Stephenson, 1979). In the recent years pesticide use has increased tremendously in Kenya, Sudan, Tanzania, Zimbabwe, Cameroon and Ivory Coast. These are countries that engage in high valued cash crop production such as floriculture, coffee, tea, cocoa, and cotton. Although tea in Kenya has been relatively pests and diseases free compared to for example, tea in India or Sri Lanka, serious outbreaks have sporadically been reported. Consequently, measures to control both pests and diseases have been reported. Numerous people commented on the association between increasing temperatures and the prevalence of pests and diseases, an issue of increasing importance to Kenya. Past years' unpredicted and unprecedented pest infestation in Rwanda was seen as a warning of what could happen in Kenya, where the tea mosquito bug (*helopeltis spp*) has recently appeared (Adaptation Workshop, 2011).

Pesticides in the developing countries are mainly available in conventional formulations such as dust, wettable powder, emulsifiable concentrates, solutions, etc. Such conventional formulations pose problems relating to environmental protection, leaving residues in the ecosystem, food, finished products, etc. (United Nations Industrial Development Organization, 2009).

When pesticides are applied to food crops they degrade through chemical and biological processes at a rate determined by the nature of the chemical and plant surface or soil in which the pesticide is placed. Therefore pesticide residues may not be present as the parent compound. Many pesticides may form metabolites that are as persistent as or more persistent than the initial chemical. This fact is recognized in establishment of tolerances and acceptable daily intake (McEwen and Stephenson, 1979). Lambda- cyhalothrin has short persistence in soil and lacks systemic effect (Pesticide residues in food, 1986).The main concern in the study of pesticide residues in food is to ensure safety of the food supply. It is reported that pesticides can cause allergies and asthma like symptoms and can affect body organs such as the liver, kidneys, and the nervous system.

The problem of residues in food has been addressed at an international level through committees sponsored by United Nations. Acceptable daily intake (ADI) has been established for a number of pesticides and presented with suggested tolerances in a series of annual reports of joint FAO/WHO meetings (McEwen and Stephenson., 1979). It was therefore important to carry out this research so as to establish what maximum residue is likely to be present in fresh tea leaves, processed tea, and brewed tea, when pesticides are used in a manner effective for pest control. In this study, field trials were set up to estimate pesticide residue levels in fresh, black and brewed tea for Karate, a broad-spectrum synthetic pyrethroid for control of foliar insect pests. Reports have shown that normal methods of food preparation significantly reduce pesticide residues (McEwen and Stephenson., 1979). Tea as a product undergoes various preparations from the fresh leaf to the black brewed tea for consumption. The extent of such effects varies with the pesticide residues and the nature of food product. The importance of these procedures in reducing pesticide residues will be established by comparing residue levels in fresh tea leaves, black tea and brewed tea.

Tea is susceptible to a number of insect and mite pests. Therefore application of pesticides such as Karate for control of the pests in tea such as thrips, aphids, and weevils is necessary. Karate is a broad-spectrum synthetic Pyrethroid insecticide used for control of biting and sucking pests in crops. It has a high level activity against a wide range of insects and it also has miticidal activity. The compound has a quick knock down and repellency effect through contact, residual and stomach activity. The chemical is relatively stable to degradation in sunlight; hence it is used as a practical tool in agriculture. Treatments of karate are effective against major pests such as boring caterpillars or leaf miners. Application should be done when insects are noticed and a spray interval of seven days observed depending on the amount of rain and pest infestation. A programme of sprays is usually required particularly during more active growth stages of the plant. Karate is applied at a maximum rate of 3.0l/Ha to mature tea bushes with shoots.

Karate is a pesticide that is foliar sprayed on tea for protection against pests such as boring caterpillars or leaf miners, mites, aphids, whiteflies, thrips and diamond back moth. Karate is commercially available in emulsifiable formulations. It occurs as a 1: 1 mixture of two enantiomeric pair of (S)-α-cyano-3-phenoxybenzyl Z- (1R, 3R)-3-(2-chloro-3, 3, 3-trifluoropropenyl)-2, 2-dimethylcyclopropane carboxylate and (R)-α-cyano-3-phenoxybenzyl Z-(1S, 3S)-3-(2-chloro-3, 3, 3-trifluoropropenyl)-2, 2-dimethylcyclopropane carboxylate. It contains approximately 90% lambda cyhalothrin and small amounts of other cyhalothrin isomers. Lambda-cyhalothrin the active ingredient has the chemical formula $C_{23}H_{19}ClF_3NO_3$ and molecular weight of 449.9 and the technical material is a viscous, oduorless, liquid. It is insoluble in water but soluble in a range of organic solvents. The structure of the two enantiomeric pairs is given below

(S) (Z)-(IR)-Cis- (S) (Z)-(IS)-Cis-

Figure 1: Structure of Lambda-cyhalothrin (Tomlin, 1997)

Most synthetic pyrethroids have α- Cyano 3- Phenoxybenzyl or a 3-Phenoxybenzyl group as the alcohol moiety and produce 3-phenoxybenzoic acid (PBA) as the ester cleavage metabolite Therefore Lambda-cyhalothrin metabolises to 3-Phenoxybenzoic acid (PBA). A study on thirteen synthetic Pyrethroid insecticides and their ester cleavage metabolite PBA in tea indicate residues of the pyrethroids were found but no 3- Phenoxybenzoic acid (PBA) were detected (Tsumura *et al*, 1994). Therefore the study analyzed the residues of the parent Pyrethroid Lambda-cyhalothrin only.

Residues of this pesticide could reach and affect consumers of tea. Therefore it is necessary to determine the levels of residues likely to appear in drinking tea. In carrying out this endeavor, it was necessary to determine pesticide concentration levels at different stages of tea preparation. This ensured that it was possible to determine the effect of processing on residue levels in tea. This study is also necessary in order to ensure correct use of pesticides on tea in terms of application rates and Pre- Harvest Interval and to permit circulation of tea in the world markets even though they are treated with pesticides as long as the residues comply with harmonized MRLs. More so, it is to ensure that pesticide residues if any remaining in tea are of acceptable levels so that there are no health risks to the consumer. Plant residues are isolated by liquid-liquid extraction (LLE) because they contain water, plant pigments, lipids, proteins, essential oils and waxes. A comparison of the various isolation and cleaning techniques for pesticide residue analysis show that LLE has a good isolation effect and it is universal for food and plant materials. Florisil column chromatography has a good isolation effect and very good cleaning effect for plant materials. Acetone has been used in LLE of synthetic pyrethroids in tea leaves. Classical LLE offers a wide choice of organic solvents for effective analyte isolation from the sample e.g. pure acetone, methanol or their mixtures with medium polar organic solvents are often used for extraction of various pesticide residues from biological matrix (Tekel *et al.*, 2001).

Residue data are required for samples of made tea in the world markets. EU and FAO/WHO require producing countries to do field trials to determine the maximum residue levels under their environments. These field experiments must reflect the proposed pesticide use with respect to formulated products, dilutions and rates, modes, number and timings of applications. Enough plots should be set out to permit sampling at intervals after the last application of Karate such as 0, 7 and 14 days so as to establish appropriate pre-harvest interval. Pre-harvesting interval is the period which must be left between application of a pesticide in the farm and the harvesting of a crop. This is to ensure that pesticide residue on the crop becomes within acceptable and safe limits for human use (Al-Agha *et al.,* 2005).

Methodology

The field experiment conducted in Timbilil estate in Tea Research Foundation of Kenya Kericho. It constituted two replicate Karate 1.75EC treated plots and one untreated control plot. Application of Karate was done at a rate of 3.0ml Karate 1.75 E.C per litre of water. No physical crop maintenance practices such as tilling, hoeing or pruning was carried out during the period of the study. The application was made to mature tea leaves as new growth appeared at the beginning of the dry season in Kericho. The application was made using a knapsack sprayer with a hand held boom as is typical commercial practice. The rate of application was done at the maximum proposed rate, intended to be a worst case treatment pattern to be used in Kenya. Sampling of leaves for residue determination was done 0, 7 and 14 days after application so as to establish the pre-harvest interval. This was to ensure that pesticide residue on the crop was within acceptable and safe limits for human use. Typical local practice were used in the harvest of tea samples including plucking with bare hands, use of rubber aprons, use of net plucking bags and ensuring plucking two leaves and a bud. Leaves were then transferred into plastic lined residue sampling bags on site and transported to TRFK processing facility and laboratory for weighing.

Fresh tea leaf samples, Black Tea Processing and Brewed Tea Preparation

Small fresh tea leaf samples not designated for processing were placed into a freezer at TRFK laboratories until extraction. Black tea processing was performed in a miniature-scale tea processing facility at the Tea Research Foundation of Kenya, Kericho. The process is designed to simulate as closely as possible the commercial black tea processing procedure that is standard in Kenya (Stefan, 1997). The tea processing includes withering which results in moisture loss. Biochemical changes also occur within the leaf matrix. Enzymes begin to gradually ferment the leaf material to add the complexity of Flavor and quality of tea. This is followed by leaf maceration a step in which the cell structure of the leaf matrix is physically destroyed to allow fermentation to occur by a process of crush, tear and curl (CTC). This process allows air to circulate into the tea matrix, where oxygen works with enzymes from the plant cells to ferment the entirerity of the matrix. Fermentation or oxidation is a process by which tea quality is achieved. The macerate leaf is placed in several trays whereby humidified ambient air is blown between the trays in order to supply oxygen to the fermentation reaction and also dissipate heat that is generated by the exothermic reaction. Tea drying terminates the fermentation process. The tea is considered dried at moisture content of approximately 2.5 to 4%. Brewed tea was prepared by weighing 20 g of black tea into a flask then 300 mL of boiling distilled water was added. The contents of the flask were allowed to stand for 5 minutes. The liquid portion was filtered through a filter paper into another flask. Another 300 mL of boiling water was added to the solid black tea remaining in the flask and allowed to stand for 5 minutes. The liquid portion was filtered into the jar containing the first liquid portion. The sample was allowed to cool to room temperature (Samantha and Dan, 1995) and the extraction was then carried out.

Sample Extraction

Hydration of the tea samples was done before extraction to ensure efficient extraction. The Lambda-cyhalothrin extaction was done using hexane: acetone mixture with subsequent drying with anhydrous sodium sulphate (Subbiah and Narayanan, 2009).

Clean-up for lambda-cyhalothrin was conducted using open preparative chromatographic columns packed with 10 g silica gel and 2 g anhydrous sodium sulphate (Na_2SO_4) placed on top of the columns (Lina *et al*., 2010).

Calibration curves

Known concentrations of the pesticides were analyzed to generate a five point calibration curve of the type stated below:

The Power curve fit: $y = bx^m$ (Samantha and Dan, 1995).

Where y = the detector response, peak height
b = y intercept
x = nanograms injected
m = the slope of the line

The standard concentrations were given in terms of nanograms injected for example if 2 µl of 0.1 ppm (0.1ng/ µL) was injected; this is equivalent to 0.2 ng.

The power curve was chosen because it gives all the concentrations as positive values including those of peak heights lower than the y-intercept of the calibration line, which would otherwise be given as negative concentration if a linear curve of the form y=ax + b is used.

Secondly, the rate at which the concentration changes is not constant because the factors responsible for the change are continually changing. These factors include plant growth, sunlight intensity and amount of rainfall. In cases where data do not follow a linear trend, an exponential or power curve fit is used (Frank and Kenneth, 1969).

Calculation of concentrations

The sample peak heights from chromatograms were used in the standard equation obtained to calculate the nanograms found for each sample. The Pyrethrins in all calculations were listed as ppm Pyagro. The ppm of the pesticides was determined from the nanograms found from the calibration equation using the following steps:

$$\text{g-final weight} = \frac{\text{g-initial sample wt} \times \text{mL-aliquot}}{\text{mL-extraction solvent}}$$

$$(\text{ng/mg})\ \text{ppm} = \frac{A \times B \times C}{D \times E}$$

A = ng- found
B = final volume, µL
C = dilution factor
D = µL- injected
E = final weight, mg

After obtaining the concentration of each sample, the mean concentration of each triplicate was determined.

Fortification recoveries

Analytical procedures are validated by fortification of control samples, results within the standard range of 70 % to 120% show acceptable accuracy (Stefan, 1997). Percentage recoveries of fortified control samples were calculated using the equation

$$\%\ \text{recovery} = \frac{\text{ppm found in fortified sample - ppm in control} \times 100}{\text{ppm fortification level}}$$

Treated sample residues were corrected upward using the percentage recoveries for each set of samples.

RESULTS AND DISCUSSION

Time of harvest and pesticide residue levels

Pesticides are generally lost by evaporation, photo degradation, rainfall and growth dilution. The latter is a process where the pesticide present in the plant tissues spreads to new tissues as the plant grows, leading to lower concentrations as the number of tissues increase. The chromatograms of 0, 7 and 14-day samples for Karate and the decay curves reveal that the pesticide residue levels decrease as the number of days after pesticide application increase (Muraleedharan *et, al* 2003).

Residue decay curves

The residue decay curves given in figures 2 and 3 show the decline in the pesticide concentration in the samples collected 0, 7 and 14 days. The figures show that the pesticides decay gradually from the time of application. It shows decay in the fresh leaves, this decay is mainly attributed to growth dilution, photo degradation and rainfall. Figure 3 show residues in black tea samples which significantly lower than the residues of the respective fresh tea samples. The difference is as a result of thermal decomposition during the processing of black tea due to high temperatures.

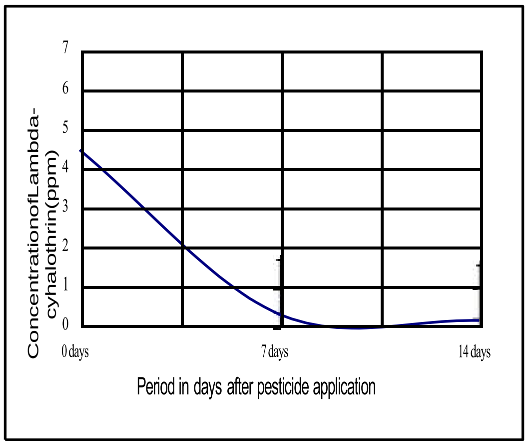

Fig 2: Karate (Lambda-cyhalothrin) Collected on 0.7 and 14 days after Pesticide application

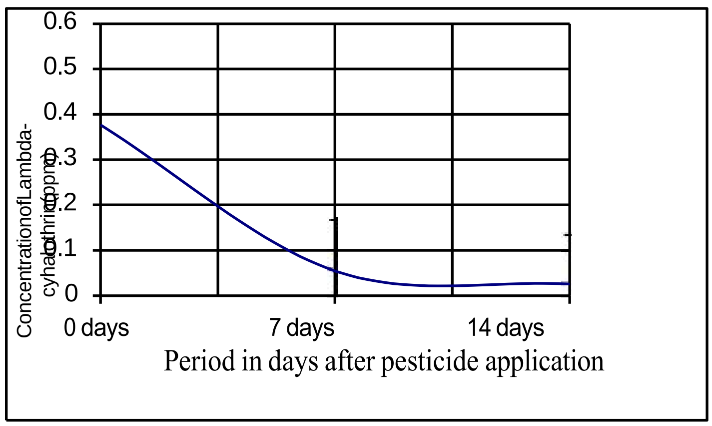

Fig 3: Karate (Lambda-cyhalothrin) concentration in black tea samples collected on 0,7 and 14 days after pesticide application

Effect of processing on pesticide residue levels

Further degradation of pesticide residues takes place due to thermal decomposition during manufacture of black tea, as the tea is exposed to high temperature. More so, during preparation of brewed tea, pesticide residues contained in black tea are further degraded by high temperature. The reduction of the pesticide residues due to processing are shown in figures 4 below. Pesticide residues may leach into the brewed tea or remain in the spent tea depending on their solubility in water.

Figure 4 shows a decline of pesticide residues as black tea is processed from the fresh leaf and as brewed tea is made from black tea. On the day karate was applied, the pesticide residues decline from 4.535 ppm in fresh leaf sample to 0.377 ppm in black tea. This is a percentage decrease of 91.7%. The residues in the samples collected 7 days after application of karate were found to be significantly lower than for the samples collected on the day of pesticide application, for the fresh leaves the pesticide concentration on the day of pesticide application was found to be 4.535 ppm and 7 days after application the concentration was found to be 0.349 ppm, a 92% decrease. The residue levels decrease further during processing from 0.349 ppm in the fresh leaf samples to 0.059 ppm in black tea (83% decrease).

The bar graph shows a steady decline of the already low lambda-cyhalothrin (Karate) residues in the samples collected 14 days after pesticide application. The decrease from 0.194 ppm in the fresh leaf samples to 0.026 ppm in the black tea, this is equivalent to 86.6% decrease.

The decrease of the residues as the fresh leaf is used in the manufacture of black tea can be attributed to thermal decomposition due to high temperatures of about 120°C during the manufacture of black tea (Stefan, 1997). The decrease of the residues in the same matrix harvested at different pre-harvest interval can be attributed to growth dilution, photo degradation and rainfall (Muraleedharan *et, al* 2003).

No residues were detected in the brewed tea and this may be attributed to the insolubility of lambda-cyhalothrin or decomposition and evaporation during brewing.

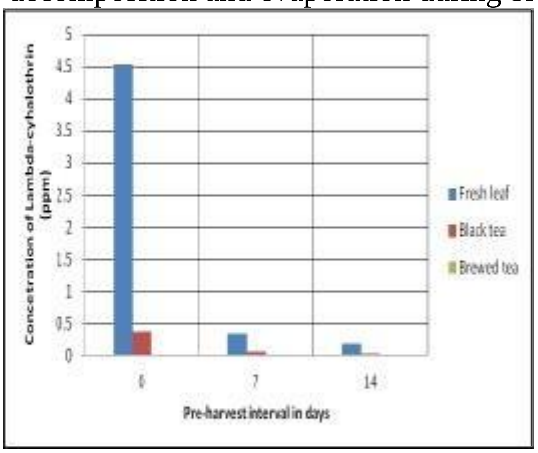

Fig 4: Concentration of Lambda-cyhalothrin in Fresh, Black and Brewed tea after 0, 7 and 14 days after application of Karate

Conclusion

The residue levels shown in the residue decay curves and the bar graph show that application of Karate in fresh leaves result in residues in the tea product and that tea preparation procedures lead to reduction of pesticide residues in the tea product. The residues found in the tea after application of the pesticide at maximum proposed rates indicate that the best time of harvesting (pre-harvest interval) after pesticide application is seven days.

The Kericho trial site was representative of diverse tea growing areas in Kenya. Karate 1.75 E.C is used in Kenya to control pests in tea. The treatment rate was intended as a maximum rate proposed for use on tea in Kenya so as to represent a worst-case treatment. The application parameters considered during the field trial may be proposed for use in Kericho.

Karate residues in the various tea matrices were higher than the method LOQ of 0.01 ppm, except for some samples of black tea taken at 14 days after pesticide application and for all the brewed tea samples that had no detectable residue levels.

Based on the results presented above; treatment of mature tea bushes with Karate 1.75EC according to the intended use pattern tested in Kericho will produce no detectable residue levels of Karate in brewed tea. Generally it will give low levels of Karate residues in fresh and black tea. The residues found in this study lie below the acceptable Maximum Residue Limits (MRLs) of 0.1ppm in dried (black) tea established within the European Union (E.U). Thus karate if used as recommended will pose no risks to the consumers of tea.

Recommendations

1. Plucking of tea should not be done before a period of seven days after spraying with Karate
2. Spraying should be done after plucking so that a minimum safe period is always maintained, that is before new shoots are available for plucking, which is approximately seven to fourteen days apart.

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