DETERMINATION OF KARATE AND PYAGRO RESIDUE LEVELS IN FRESH, BLACK AND BREWED TEA FROM TEA FARMS IN KERICHO

By

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted for an award in any institution as by my knowledge.

Sign:_____ Date:_____

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RECOMMENDATION

This thesis has been presented for examination with our approval as the candidate's University supervisors.

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DEDICATION

This work is dedicated to my loving husband Elly Kipng'etich Koross and my son Crispin Chirchir.

ABSTRACT

Karate and Pyagro are insecticides currently registered and used in Kenya for control of several insect and mite pests in diverse crops. They are emulsifiable concentrate formulations of the active ingredients lambda-cyhalothrin (Karate) and Pyrethrins (Pyagro). This study established residue levels for lambda-cyhalothrin and Pyrethrins 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 monitors the decline of pesticide residues under normal harvest time intervals. The samples were collected at various intervals after application of pesticides at maximum proposed application rates (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. Two replicate plots of 6 by 15 tea bushes were treated with Karate 1.75 EC, another two replicate plots of 6 by 15 tea bushes were treated with Pyagro 4 EC and one untreated plot of 6 by 20 tea bushes at the field trial. Foliar applications were made to mature tea bushes, Karate was prepared by dissolving 3.0 mL of Karate concentrate in a litre of water and Pyagro was prepared by dissolving 5.0 mL of Pyagro concentrate in a litre of water.

Extraction of lambda-cyhalothrin from Karate treated samples was accomplished using 50% acetone in hexane and Pyrethrins from Pyagro treated samples were extracted using acetonitrile then partitioned with petroleum ether. Analysis of the samples was done by Gas Chromatography (GC), employing the electron capture detector with a packed column. 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 results reveal that residues found in samples collected on the first day after application contain the highest residue levels and those collected fourteen days after application contain the lowest residue levels. The processing and brewing of tea appear to affect the residues of lambda-cyhalothrin (Karate) and Pyrethrins (Pyagro) most significantly i.e. for the same pre-harvest interval, the residues in fresh tea samples are higher than residues in the black tea samples which are also higher than the residues in the brewed tea samples. The study determined the maximum residue level and compared it with the suggested tolerances. The residue levels from the study are lower than the maximum residue levels allowed within the European Union. Therefore, if these pesticides are used according to the established pattern they will pose no risk to the consumers of tea.

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LIST OF ABBREVIATIONS

µL	-Microlitres
1.75 EC	- 1.75g/100ml Emulsifiable Concentrate
4 EC	- 4g/100ml Emulsifiable Concentrate
ADI	- Acceptable Daily Intake
AOAC	- Association of Official Analytical Chemists
ARfD	- Acute Reference Doses
CTC	- Crush Tear and Curl
Dil	-Dilution
EU	- European Union
FAO	- Food and Agricultural Organization
Fin. vol.	- Final volume
Fortif	- Fortification
GC	- Gas Chromatography
GLC	- Gas Liquid Chromatography
Inj	-Injected
LLE	- Liquid-Liquid Extraction
LOQ	- Limit of Quantitation
MGK	-McLaughlin Gormley King
MW	- Molecular Weight
MFK	- Manufactured Karate
MFKB	- Manufactured Karate Brewed
MFKBF	- Manufactured Karate Brewed Fortified
MFKF	- Manufactured Karate Fortified
MFPyagro	- Manufactured Pyagro
MFPyagro B	- Manufactured Pyagro Brewed
MFPyagro BF	- Manufactured Pyagro Brewed Fortified
mg	- milligrams
MRLs	- Maximum Residue Levels
NADPH	- Nicotinamide Adenine Dinucleotide Phosphate

ND	-Not Detected
ng	- nanograms
PF	- Pyagro Fortified
PHI	- Pre Harvest Interval
TRFK	- Tea Research Foundation of Kenya
USEPA	- United States Environmental Protection Agency
UV	- Ultra Violet
WHO	- World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background

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 *et al*, 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.

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 *et al,* 1979). Lambda- cyhalothrin has short persistence in soil and lacks systemic effect. (FAO, 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 *et al,* 1979). It was therefore important to carry out this research so as to establish what maximum residue is likely to be present in raw agricultural commodity i.e. 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 two of the most commonly used pesticides in the tea industry in Kenya. These pesticides are Karate, a broad-spectrum synthetic Pyrethroid and Pyagro, a natural Pyrethrins formulations for control of foliar insect pests. The study includes determination of residues in fresh tea leaves, black tea and brewed black tea to evaluate the effect of various tea preparation procedures on pesticide residue levels in tea.

Reports have shown that normal methods of food preparation significantly reduce pesticide residues (McEwen F. L *et al* 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 and Pyagro for control of the pests such as tea thrips, tea aphids, and tea 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 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.

Pyagro is a broad spectrum insecticide recommended for control of mites, aphids, thrips and diamond back moth. The chemical is applied as a full cover spray on foliage by any ground applicator. Application should be done when insects are noticed and a spray interval of seven days observed. Shorter spray intervals can be used in case of heavy pest infestation. Spraying of Pyagro should be done early in the morning or late in the afternoon when the effect of UV radiation is low. A 5l/Ha is the maximum proposed rate on tea.

1.2 Statement of the problem

Karate and Pyagro are pesticides that are foliar sprayed on tea for protection against pests such as boring caterpillars or leaf miners, mites, aphids, whiteflies, thrips and diamond back moth. Residues of these pesticides 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.

1.3 Hypotheses

1) Application of Karate and Pyagro in fresh leaves result in unacceptable residues in the tea product.
2) Tea preparation procedures, lead to reduction of the pesticide residues in the tea product.

1.4 Objectives

1) To determine the maximum residue levels (MRLs) in tea, after application of the pesticides at maximum proposed application rates.
2) To determine the Pre-harvest interval (PHI) that is, safe time of harvesting, after pesticide application by monitoring the decline of residue levels under normal harvest time.
3) To establish the role of tea processing and preparation in reducing pesticide residue levels.

1.5 Justification

The study aimed at determining the residue levels of Karate and Pyagro in tea which is necessary to minimize exposure of consumers of tea to harmful levels of pesticides. This research is also necessary in order to control 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.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of pesticide

The word pesticide is a blanket term, which covers any chemical or agent used for pest control. They are grouped based on the type of pest controlled. The three main groups are; fungicides used in control of fungal spores, herbicides for weed control, and insecticides for control of insects. Others include nematicides for control of worms, molluscicides for control of slugs and snails, acaricide for control of mites, and rodenticides for control of rodents (Heaton, 1994).

2.2 Residue studies in plants

Spices and medicinal plants are used as raw materials for pharmaceutical preparations and as supplements for dietetic products. These plants are susceptible to insect and disease attacks and thus pesticides are widely used for their control. Published research on pesticide residues in crude herbal material indicates presence of chlorinated pesticide residues. Other potentially contaminating pesticides include organophosphates, carbamate insecticides and herbicides (Abou Arab and Abou M.A, 2001). Government agencies require data for pesticide residues to delineate the nature and amount of residue in crops, byproducts, and processed products, which could be used for food or feed purposes (McEwen and Stephenson, 1979).

In studying plant residues they 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 Jozef *et al*, 2001).

2.3 Field experiment

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 both Karate and Pyagro 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).

Exceeded MRLs indicate violations of good agricultural practice (GAP) i.e. the recommended field activities on the crop such as pruning, hoeing and the correct use of pesticides in terms of authorizations or registration granted.

2.4 Commonly used pesticides in tea

Pyrethroids comprise an important group of insecticides and are widely used in many countries (Yukari, 1994). Agriculture is the largest user of pesticides especially for insect, plant disease and weed control. The most commonly used pesticides in the tea industry in Kenya include Karate a synthetic Pyrethroid and Pyagro a natural Pyrethrin for control of foliar insect pests.

2.4.1 Methods of analysis of Karate (Lambda-cyhalothrin)

The method of crop residue analysis for cyhalothrin, which is also applicable to lambda-cyhalothrin, was reviewed by the 1984 FAO meeting. The crops were prepared for analysis by mincing or chopping until a homogeneous sample is obtained. Samples were extracted using 50% acetone in hexane and extracts washed with water. Co-extractives were removed by liquid-liquid partition chromatography where necessary. All crop extracts were cleaned using florisil column chromatography. Final quantitative determination of cyhalothrin was by gas liquid chromatography using electron capture detector. The limit of detection for total cyhalothrin isomers is 0.01mg/kg. (FAO, 1986).

2.4.2 Methods of analysis of Pyagro (Pyrethrin)

Pyrethrum used alone or synergized is registered by the United States Environmental Protection Agency (USEPA) for the control of pests under diverse conditions. (Casida, 1995). Some uses of pyrethrum namely those associated with protection of food, feed and food animals from insect attack require the establishment of residue tolerances. Environmental protection agency (1986) as part of its data call-in program requested that residue studies be conducted to support all areas of uses requiring tolerances. This issue was addressed by the pyrethrin steering committee, and the technical sub-committee reviewed existing data, found out it was unsatisfactory and initiated studies from the use of pyrethrum in food processing, growing crops (which covers tolerances on raw agricultural commodities), milk, eggs and meat animals. (Casida, 1995).

Pyrethrum contains both Pyrethrin I (consisting of Pyrethrin I, Cinerin I and Jasmolin I) and Pyrethrin II (Pyrethrin II, Cinerin II and Jasmolin II), which are typically present in similar amounts and represent the major insecticidal components of the mixture. The quantification of small amounts of pyrethrin residue has historically been based on pyrethrin I due to thermal instability and decomposition of pyrethrin II under gas Chromatographic conditions.

The primary quantitation methodology of world trade of crude oleoresin and refined extract today is Association of Official Analytical Chemists (AOAC) titrimetric procedure, and as reproduced by both industry and government regulators. This method is not suitable for analysis of residue levels of pyrethrins. The conjugation of the ketone to the double bond on the cyclopentyl ring result in good detection by GLC using an electron capture (EC) detector. This methodology has been used extensively in pyrethrins residue analysis work. (Casida, 1995)

Meinen and Bergman (1991), McLaughlin Gormley King (MGK) senior research associate and Analytical chemist, respectively, developed and used the following procedure for the residue analysis of pyrethrins in food with the following Gas Chromatographic conditions.

The instrument used was a Varian type fitted with an electron capture detector and 120 cm x 4 mm id, 3% X_e-60 chromosorbs W (HP), 80-100-mesh column. The carrier gas used was argon /methane flowing at a rate of 50 ml/min. Temperatures were set at 180^0C for the column, 300^0C for the injector and 300^0C for the detector. The limits of detection are 0.05 ppm for the total pyrethrins I.

Below is the schematic method for analysis of pyrethrins residues in food commodities and in crops.

Food or crop sample ⟶ Acetonitrile extraction ⟶

Acetonitrile/petroleum ether partition ⟶ Florisil clean up ⟶ electron capture (Casida, 1995).

2.4.3 Metabolites of Lambda-cyhalothrin

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

Studies on the fate of residues in plants were carried out on cotton, cabbage leaves, apples and peaches indicate that residues of the metabolite 3-Phenoxybenzoic acid (PBA) were very much less than the lambda- cyhalothrin residues. (FAO, 1986). Therefore the study analyzed the residues of the parent Pyrethroid Lambda-cyhalothrin

2.4.4 Metabolites of Pyrethrins

Pyagro as a pesticide is quite stable in air but rapidly breaks down in presence of sunlight, it also degrades at high temperatures above 70^0 C as well as in contact with alkaline medium or alkaline materials such as ammonia, lime etc. Low temperature storage of bulk crude extracts may cause deposits of plant waxes without necessarily affecting the pyrethrins content. (Wangai and Kitazi, 2001).

The ester linkage of natural Pyrethroids is unstable when exposed to enzymes of the insect gut; therefore they are often destroyed by oxidation rather than hydrolysis.The methyl group on the isobutenyl side chain of the Crysanthemic acid is oxidized to hydroxymethyl group in a NADPH- dependent reaction. The primary alcohol group formed in this way from Pyrethrin I is readily converted to a carboxylic acid group. Pyrethrin II is metabolized in a similar way. (Kenneth *et, al,* 1982). Oxidative changes can also take place in the five-carbon atom side chain of the alcohol moiety; altogether some ten metabolites have been recognized. (Yamamoto *et, al,* 1971)

Since metabolites of Pyrethrin I and II are likely to be less toxic than Pyrethrins I and II (Elliot *et, al* 1972), the Pyrethrin steering committee technical sub-committee decided to analyze plant tissue for residues of Pyrethrins only. Therefore in this study, residues of Pyrethrins only not its metabolites were investigated.

2.5 Pesticide health risks

Poisoning and deaths can result due to misuse and mishandling of pesticides. Studies have revealed that most farmers do not put on protective wear, smoke and eat during application of pesticides. These have led to complain of health problems such as headache, coughing, skin rashes and breathing difficulties by farmers. (Al-Agha *et al* 2005). Issues such as disposal of the pesticide containers and pre-harvest interval are of dangerous impacts to the general public. Toxicity of pesticides is variable from one type to another. The risks of pesticides to humans depend on the exposure and toxicity. Long periods of exposure are dangerous even if the toxicity low.

Pesticide residue levels are regulated through Maximum Residues Levels (MRLs), Acceptable Daily Intake (ADI) and Acute Reference Doses (ARfD) so as to minimize exposure of consumers to harmful pesticide intake and to control the correct use of pesticides in terms of authorizations or registration granted on application rates and pre harvest interval. If MRLs are exceeded Acceptable Daily Intake (ADI) and/or Acute Reference Doses (ARfD) will indicate whether or not there is possible chronic or acute health risk respectively. (European Commission, 2004).

Some studies suggest increase in incidences of cancer could be due pesticides application. Pesticides and industrial chemicals are known to activate the formation of toxic intermediates in organisms. A number of pesticides are not considered direct carcinogens but in the human organism, carcinogenic metabolites are formed due to metabolism. More so, there exist combined effects of two or more pesticides. (Avagyan *et al*, 2005). Pyrethrins have been classified as moderately hazardous by WHO and the acceptable daily intake given as 0.04mg/kg body weight. (Wangai and Kitazi, 2001)

2.6 Pesticides mode of action

2.6.1 Mode of action of Karate

Karate whose active ingredient is Lambda-cyhalothrin, is a synthetic Pyrethroid insecticide with a high level of activity against a wide range of Lepidoptera, Hemiptera, Diptera and caleoptera spp. It is a stomach, contact and residual insecticide. It is relatively stable to degradation in sunlight. It is not plant systemic (i.e. it is not translocated within the plant system), has very little fumigant or translaminar activity and has short persistence in soil. (FAO, 1986)

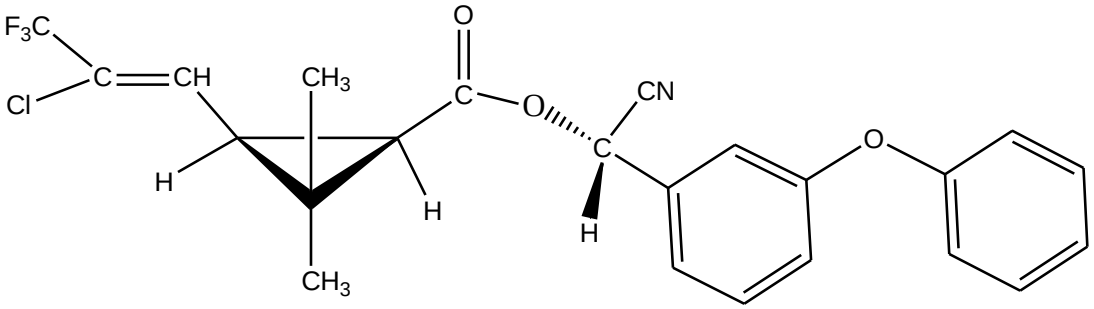
2.6.2 Mode of action of Pyagro

Pyagro is abroad spectrum natural Pyrethrin insecticide recommended for control of aphids, mites, whiteflies, thrips and diamond back moth. It acts by contact bring about quick knock down, paralysis and kill of insects. It has a rapid action and strong repellency achieved within two hours after spray. It degrades at high temperatures above 70^0 C (Wangai, and Kitazi, 2001)

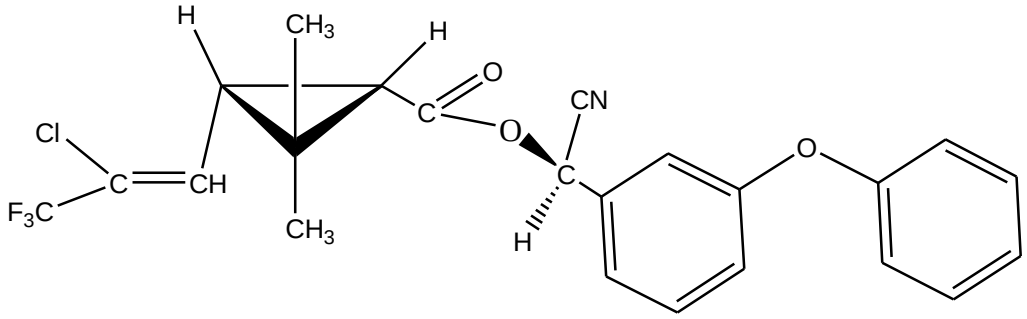
2.7 Test substances

2.7.1 Karate 1.75 E.C (Lambda-cyhalothrin)

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 2.1: Structure of Lambda-cyhalothrin (Tomlin, 1997)

2.7.2 Pyagro 4EC (a natural pyrethrin formulation)

Pyagro is a natural Pyrethrin formulation containing 4% Pyrethrins. Pyrethrum extracts contain three naturally occurring closely related insecticidal esters of Chrysanthemic acid (Pyrethrin I) and three other esters of Pyrethric acid (Pyrethrin II) and three alcohols; Pyrethrolone, Cinerin and Jasmolin. The sum of Pyrethrins I and II collectively designated, as "the Pyrethrins" constitute 45-50% of the pyrethrum extract. (Casida, 1995). Pyrethrins therefore occurs as a mixture of six related esters which are, Pyrethrin I (M.W = 328.4) Cinerin I (M.W 316.4) Jasmolin I (M.W 330.4) Pyrethrin II (M.W = 372.4) Cinerin II (M.W = 360.4) and Jasmolin II (M.W = 374). The formulation occurs as a dark viscous liquid. It is insoluble in water and soluble in organic solvents

Pyrethrin I esters of Chrysanthemic acid (Chrysanthemates)

	R	R^1
Pyrethrin I	CH_3	$CH=CH_2$
Cinerin I	CH_3	CH_3
Jasmolin I	CH_3	CH_2CH_3

Pyrethrin II –esters of pyrethric acid (Pyrethrates)

	R	R^1
Pyrethrin II	$CH_3OC(O)$	$CH=CH_2$
Cinerin II	$CH_3OC(O)$	CH_3
Jasmolin II	$CH_3OC(O)$	CH_2CH_3

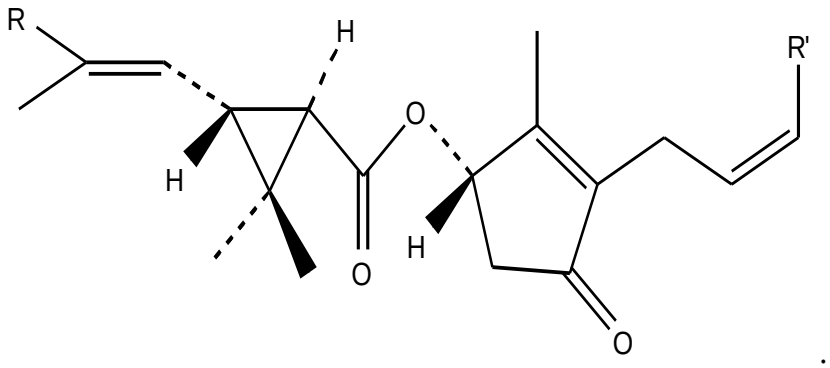


Figure 2.2: Structure of Pyrethrin (Casida, 1995)

2.8 Black tea processing

Black tea processing was performed in a miniature-scale tea processing facility at the Tea Research Foundation of Kenya headquarters, Kericho. The process is designed to simulate as closely as possible the commercial black tea processing procedure that is standard in Kenya. The processing of fresh tea leaf samples into black tea can be summarized as follows (Stefan, 1997):

2.8.1 Withering

In this step, fresh leaves are placed within a trough and ambient temperature air is blown through the leaves for approximately 14 to 18 hours. This involves both physical and chemical

changes of the leaf matrix. Physical withering involves the loss of moisture, which will make cell walls more pliable and the tea easier to macerate. 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

2.8.2 Maceration

This is a step in which the cell structure of the leaf matrix is physically destroyed to allow fermentation to occur. To achieve maceration, withered tea is gradually introduced into a crush, tear and curl (CTC) machine in which two sharply serrated rotating rollers rolling in different directions at different speeds rips tea leaves into smaller particles and twists, rolls and crushes the smaller particles. 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.

2.8.3 Fermentation

Fermentation is allowed to progress until appropriate tea quality is achieved. In fermentation the macerate leaf is placed in several trays or racks within a fermentation chamber in which humified ambient air is blown between the trays of fermenting leaves. The air supplies oxygen to the fermentation reaction and also dissipates heat that is generated by the exothermic reaction. This reaction is allowed to proceed for about 90 minutes.

2.8.4 Drying

This step terminates the fermentation process. In Kenya drying of the black tea is performed within a fluid bed drier, which is a cylindrical chamber in which hot air is blown upwards through perforations in the bottom into the drying tea. The drying tea gradually becomes suspended in air while being continuously blown upward reaching an equilibrium simulating a fluid bed. The tea is considered dried at moisture content of approximately 2.5 to 4%.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Field trial procedures

The field portion of this study was conducted at the Tea Research Foundation of Kenya in Kericho, Timbilil Estate where the applications were done according to good agricultural practice (GAP) i.e. the application was carried out according to the label recommendations on the rates and safety measures to be observed. This was in order to determine the maximum residues likely to be present in fresh, dried and brewed tea product.

The trial site included two replicate Karate 1.75 EC treated plots, two replicate Pyagro 4 EC treated plots and one untreated control plot. The treated and control plots all encompassed several rows of mature tea bushes. All treated plots were 6 by 15 bushes of tea and the untreated plot 6 by 20 bushes of tea. To minimize contamination buffer zones were left between treated and untreated plots and also around the border of all plots.

At the trial site two replicate plots were treated once with one rate of karate 1.75 EC and the other two replicate plots treated with one rate of Pyagro 4 EC on the same date. Application of karate made at a rate of 3.0 ml karate 1.75 EC per litre of water. Application of Pyagro was made at a rate of 5.0 ml Pyagro 4 EC per litre of water. No physical crop maintenance practices such as tilling, hoeing, or pruning was carried out during the period of the study.

Application of the two pesticides was made to maturing tea leaves as new growth appeared at the beginning of the dry season in Kericho. Karate and Pyagro were applied after mixing with water only. Applications were made in a manner that simulated the coverage and manner of deposition of an application made in commercial tea cultivation. Applications were made using a knapsack sprayer with a handheld boom, as is typical commercial practice. The application of the pesticides were done at the maximum proposed rate, intended to be a worst-case treatment pattern to be used on tea in Kenya.

3.2 Sampling

Harvest of fresh tea leaf samples was conducted at three intervals following the first and final application. This was done at 0, 7 and 14 days pre-harvest intervals. The tea leaves were hand plucked into net sacks. Typical local practices were used in the harvest of tea samples including plucking with bare hands, use of rubber aprons and use of net plucking bags.

Appropriate measures were taken such as plucking untreated samples first and perform all field operations away from treated areas. Leaves were then transferred into plastic lined residue sampling bags on site and transported to TRFK processing facility and laboratory for weighing. Small fresh tea leaf samples not designated for processing were placed into a freezer at TRFK laboratories until analysis.

At different sampling times (0, 7 and 14days) after pesticide application, small fresh tea leaf samples were taken from all plots: two from each treated plot and one from untreated plot. Also at least 2kg leaf samples were taken, from the different plots for processing at the miniature tea factory at TRFK headquarters on the same day of harvest.

Fresh tea leaf samples from the treated plots for each pesticide (Karate and Pyagro) were thoroughly mixed to obtain a homogenous sample, which was then sub-sampled into three replicate samples for analysis. The samples were held in frozen storage at the TRFK laboratory facility until analysis (extraction). The processed samples were maintained in storage at TRFK headquarters Kericho prior to transportation to the analytical laboratory at Pyrethrum Board of Kenya Nakuru, together with frozen leaf samples.

The samples collected were analysed using methods developed from previous works on Lambda-cyhalothrin and pyrethrin residues in plant materials. Hydration of the tea samples was done before extraction to ensure efficient extraction. The isolation of the analyte was done using liquid-liquid extraction (LLE). Clean up was done by florisil column chromatography, which gave good cleaning effect.

3.3 Materials

3.3.1 Reagents

Analytical grade reagents were used during the study. These included; Hexane, Acetone, Acetonitrile, Petroleum ether, anhydrous Sodium Sulphate, Florisil, Karate 1.75 E.C, Pyagro 4.0 E.C, distilled water, White spot Nitrogen, and Hydrogen

3.3.2 Apparatus

They include: Sample bottles (15 ml), rotary evaporator, separating funnels, filter funnels, volumetric flasks, filter papers, micro syringes, Unicam 610 Gas Chromatograph equipped with an Electron Capture detector, Packed column (OV225 + Apiezon), Computer.

3.4 Sample preparation

3.4.1 Fresh tea leaves and black tea

Preparation of fresh tea leaves included chopping until a homogeneous sample was obtained. No sample preparation procedure was necessary for black tea samples due to the small particle size.

3.4.2 Brewed tea

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 distilled 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 then allowed to cool to room temperature. (Samantha *et, al*, 1995).

3.4.3 Fortification of samples

Extraction efficiencies of pesticides are measured by addition of known amounts of the pesticide to untreated sample of the crop prior to extraction. Each sample set included at least one unfortified control, at least two fortified controls and experimental samples. Controls were fortified prior to extraction.

3.5 Preparation of analytical standards

3.5.1 Fortification standards

3.5.1.1 Karate

The amount of the standard used was adjusted to correct to 100% purity. Karate contains 17.5 g/L of lambda-cyhalothrin. Therefore 5.7 mL of karate was dissolved in Hexane and diluted to 100 ml to give 1000 ppm of lambda-cyhalothrin. Dilutions of the stock solutions were made to get 1ppm and 0.01 ppm for fortification of samples.

3.5.1.2 Pyagro

The amount of the standard used was adjusted to correct to 100% purity. Pyagro contains 40 g/L of Pyrethrins. Therefore 2.5 mL of Pyagro was dissolved in Acetonitrile and diluted to

100ml to give 1000 ppm of Pyrethrins. Dilutions of the stock solutions were made to get 5 ppm and 0.05ppm for fortification of samples.

3.5.2 Gas Chromatography calibration standards

3.5.2.1 Karate

The amount of the standard used was adjusted to correct to 100% purity. Karate contains 17.5 g/l of lambda-cyhalothrin. Therefore 0.57 mL was diluted with hexane to prepare 100 ppm of lambda-cyhalothrin. This stock solution was kept tightly stoppered and stored in a freezer. Appropriate dilutions of the stock solution were made to provide analytical curve standards to generate a five-point curve. (Samantha *et, al*, 1995)

3.5.2.2 Pyagro

The amount of the standard used was adjusted to correct to 100% purity. Pyagro contains 40 g/l of Pyrethrins. Therefore 0.25 mL was diluted with hexane to prepare 100 ppm of Pyrethrins. This stock solution was kept tightly stoppered and stored in a freezer. Appropriate dilutions of the stock solution were made to provide analytical curve standards to generate a five-point curve. (Samantha *et, al*, 1995)

3.6 Analysis of fresh tea leaves and black tea

3.6.1 Determination of Karate residues

Sample Extraction

1. A sample (fresh tea leaves or black tea) weighing 20 g was placed into a jar. Appropriate samples were fortified at this time. About 110 mL of distilled water was added to black tea and about 20 ml to fresh tea leaves, to rehydrate the sample. The jar was capped and the sample was swirled to mix and allowed to stand for approximately 10 minutes. Addition of 100 mL of hexane and 100 mL of acetone was done and the mixture blended for 3 minutes to agitate the sample completely.
2. The jar was removed from blender, covered and left to stand for approximately 5 minutes. Most of the hexane layer separated to the top. The blender was thoroughly cleaned by rinsing blades with distilled water and acetone after each sample blending.

3. A large filtering funnel with 12.5 cm whatman filter paper was set up over a 1-liter separatory funnel. Most of the hexane layer was decanted into the filter paper. After most of the hexane had filtered into the separatory funnel, the remainder of the sample homogenate was added to the filter paper and covered with aluminum foil to reduce loss of solvent. The jar was not rinsed with additional solvent.
4. Gravity filtration was continued until the filtrate dripped very slowly or ceased to drip. The filter paper or filtered solids were not rinsed with any solvent.
5. The filter paper and solids were removed and discarded but the funnel was left. The filtrate was washed with approximately 200 ml of distilled water by pouring it through the funnel rapidly to cause contents to mix adequately.
6. The hexane layer was left to separate to the top of mixture for approximately 10 to 15 minutes. The lower aqueous layer was drained and discarded. The hexane layer was kept in the separatory funnel and washed again with approximately another 200 mL of distilled water.
7. Transfer of 50 mL of the hexane layer was done through a small funnel with a filter paper and 25-30g of sodium sulphate into a 100 mL graduated mixing cylinder.
8. The sample was concentrated to approximately 2 mL on a rotary evaporator. The water bath was maintained at a temperature of 40^0C or less.
9. The concentrated hexane extract was quantitatively transferred to a test tube premarked 5 ml using several small hexane rinses. The sample was then ready for florisil clean up. (Samantha *et, al,* 1995).

3.6.2 Determination of Pyagro residues

Sample extraction

1. A sample (fresh tea leaves or black tea) weighing 20 g was placed into a jar. Appropriate samples were fortified at this time. About 110 mL of distilled water was added to black tea and 20 mL to fresh tea leaves to re-hydrate the samples.
2. The jar was capped and swirled to mix the sample and the mixture was allowed to stand for about 10 minutes. 200 mL of Acetonitrile was added and blended for 3 minutes to agitate the sample completely.

3. A large filtering funnel with a filter paper was set up over a 1 litre separatory funnel. Most of the Acetonitrile layer was decanted into the filter paper. After most of the Acetonitrile had been filtered into the funnel, the remainder of the sample homogenate was added to the filter paper and covered with aluminum foil to reduce loss of solvent.
4. Gravity filtration was continued until the filtrate dripped very slowly or ceased to drip. The filter paper was removed and discarded but the funnel was not removed.
5. The Acetonitrile layer was allowed to separate to the top and the lower layer was discarded.
6. Extraction of 50 mL portion of Acetonitrile solution was done twice, using 50 mL of petroleum ether. The Acetonitrile layer was discarded.
7. The combined petroleum ether extract was poured through a filter funnel with a filter paper containing 20-30 g of sodium sulphate into 100 mL graduated mixing cylinder.
8. The sample was concentrated to approximately 2 mL on a rotary evaporator.
9. The concentrated petroleum ether extract was quantitatively transferred into a test-tube pre-marked 5 mL using small rinses of petroleum ether, the sample was ready for Florisil clean-up (Casida, 1995).

3.7 Analysis of brewed tea

3.7.1 Determination of Karate residues

Sample Extraction

1. Fortification of appropriate sample was done to the filtered brewed tea.
2. Addition of 200 ml of acetone and 200 mL of hexane to the sample was done and the mixture agitated for 5 minutes.
3. The entire sample was poured from glass jar to a 2-liter separatory funnel and the layers were allowed to separate for about 10 minutes.
4. Approximately 200 mL of the lower aqueous layer was poured into the glass jar used for extraction.
5. Addition of 200 mL of distilled water to the 2-liter separatory funnel was done.
6. The 200 ml aliquot from the glass jar was then added to the 2-liter separatory funnel. The layers were allowed to separate for about 10 minutes.
7. Approximately 200 mL of the aqueous phase was then poured to waste.

8. Another 200 ml of distilled water was added to the 2-liter separatory funnel. The layers were allowed to separate for about 10 minutes and the entire aqueous phase was poured to waste.
9. The remaining hexane layer was poured through a 12.5 cm filter containing 30-36g of Sodium Sulphate into a 250 mL graduated mixing cylinder.
10. The filtrate is capped and mixed; 150 mL of the extract was then transferred to a 500 mL boiling flask.
11. The extract was concentrated to approximately 5 mL using a rotary evaporator.
12. The sample was then ready for florisil clean up. (Samantha *et, al*, 1995).

3.7.2 Determination of Pyagro residues

Sample extraction

1. Fortification of appropriate samples was done to the filtered brewed tea.
2. Addition of 400 ml of acetonitrile to the sample was done and the mixture agitated for 5 minutes.
3. The entire sample was poured from glass jar to a 2 litre separatory funnel. The layers were left to separate for about 10 minutes.
4. Approximately 500 mL of the lower aqueous layer was poured into the glass jar used for extraction.
5. The layers were left to separate for about 10 minutes and the entire aqueous layer was poured to waste.
6. The Acetonitrile solution was extracted twice using 200 ml portions of petroleum ether.
7. The Acetonitrile layer was discarded and the petroleum ether extract was poured through a filter paper containing 20-30 g of Sodium Sulphate.
8. Transfer of 150 mL of the extract into a 500 mL boiling flask was done and then concentrated using a rotary evaporator.
9. The concentrated extract was quantitatively transferred into a test tube premarked 5 mL using small rinses of petroleum ether. This extract was then ready for Florisil clean up. (Samantha *et, al*, 1995).

3.8 Florisil column clean up

3.8.1 Karate samples

1. A chromatographic column with a glass wool plug at the bottom was prepared. The stop cork was fully opened. Approximately 10 g of activated Florisil (Florisil was activated by putting it in the oven for at least five hours at temperatures between 100^0C and 120^0C) was added to the column and the sides were gently tapped to settle the Florisil.
2. Sodium Sulphate was added to the top to form approximately 1 cm and the column was prewashed with 50 mL Hexane. When the hexane had drained to approximately 0.5 cm from the top of Sodium Sulphate, 5 mL sample extract in hexane was added.
3. The test tube was rinsed with about 5 mL hexane and the solution was added to the column. When the hexane had drained to 0.5 cm from the top of Sodium Sulphate, the test tube was rinsed with another 5 mL of Hexane and added to the column.
4. The column was eluted with 150 mL of 5 % Acetone in Hexane and the column was left to run dry.
5. The extract was concentrated to approximately 5 mL to 10mL on a rotary evaporator, keeping temperature below 40^0C. This extract was then ready for GC analysis. (Samantha *et, al*, 1995).

3.8.2 Pyagro samples

1. A chromatographic column with a glass wool plug at the bottom was prepared. The stop cork was fully opened. Approximately 10 g of activated Florisil was added to the column and the sides were gently tapped to settle the florisil.
2. Sodium Sulphate was added to the top to form approximately 1 cm and the column was prewashed with about 50 mL of petroleum ether. When the petroleum ether had drained to approximately 0.5 cm from the top of sodium Sulphate, 5 mL sample extract was added.
3. The test tube was rinsed twice with 5 mL portions of Petroleum ether and the rinses were added to the column.

4. The column was eluted with 150 mL of Petroleum ether and the column was left to run dry.

5. The extract was concentrated to approximately 5 mL to 10 mL on a rotary evaporator, keeping temperature below 40^0C. This extract was then ready for GC analysis. (Samantha *et, al*, 1995)

3.9 Gas Chromatographic analysis

The retention time of karate and Pyagro were determined before analysis of the samples. The retention time of lambda-cyhalothrin was determined by running karate pesticide as pure lambda-cyhalothrin was not available. The retention time of Pyrethrins was determined by running pyrethrin standard prepared by the pyrethrum board of Kenya, Nakuru.

During Gas chromatographic analysis of the samples; three treated samples, two fortified samples and a control were analyzed for each preharvest interval. Before analysis of the analyte a standard was run. 0.002 mL of the standard was injected twice followed by 0.002 mL of the unknown then another injection of the standard. This was done throughout the chromatographic analysis. However in some cases where the unknown concentration was too low, the injection volume was increased for both the standard and samples to increase sensitivity. Therefore in some cases 0.003 mL, 0.004 mL, or 0.006 mL was injected. In the analysis a Unicam 610 series G.C fitted with an electron capture detector was used. The limit of quantitation of Karate is 0.01ppm (FAO, 1986), and for Pyagro is 0.05ppm (Casida, 1995).

Below is a summary of the instrumental conditions:

Instrument:	Unicam 610 series G.C
Column:	120cm X 4mm id packed with 2% (0V225 + Apiezon in ratio 3:1)
Detector:	Electron capture detector (Ni 63).

Parameters

Detector temperature:	270^0C
Column temperature:	230^0C
Injector temperature	250^0C
Carrier gas:	Nitrogen white spot
Gas flow rate:	100mL/minute
Chart speed:	0.5 cm/minute

CHAPTER FOUR

RESULTS AND DISCUSSION

The analysis of three different matrices of tea samples for residues revealed consistent residue levels in replicate samples except for a few samples, which gave inconsistent values. The deviation could be attributed to sample handling procedures, which include contamination during sample collection, transportation or extraction and analysis errors. The residues found in all the samples are given in the residue data sheets in appendix 20 and 21. Mean residues were obtained for each triplicate set of samples and were used in the discussion of results.

4.1 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: $y = bx^m$ (McKenzie Laboratories, 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 A. P. *et al*, 1969)

4.1.1 Karate calibration curve

Table 1: Karate calibration data

Nanograms injected	Peak height
0.02	0.225
0.2	2.027
1	10.771
2	24.527
10	88.3

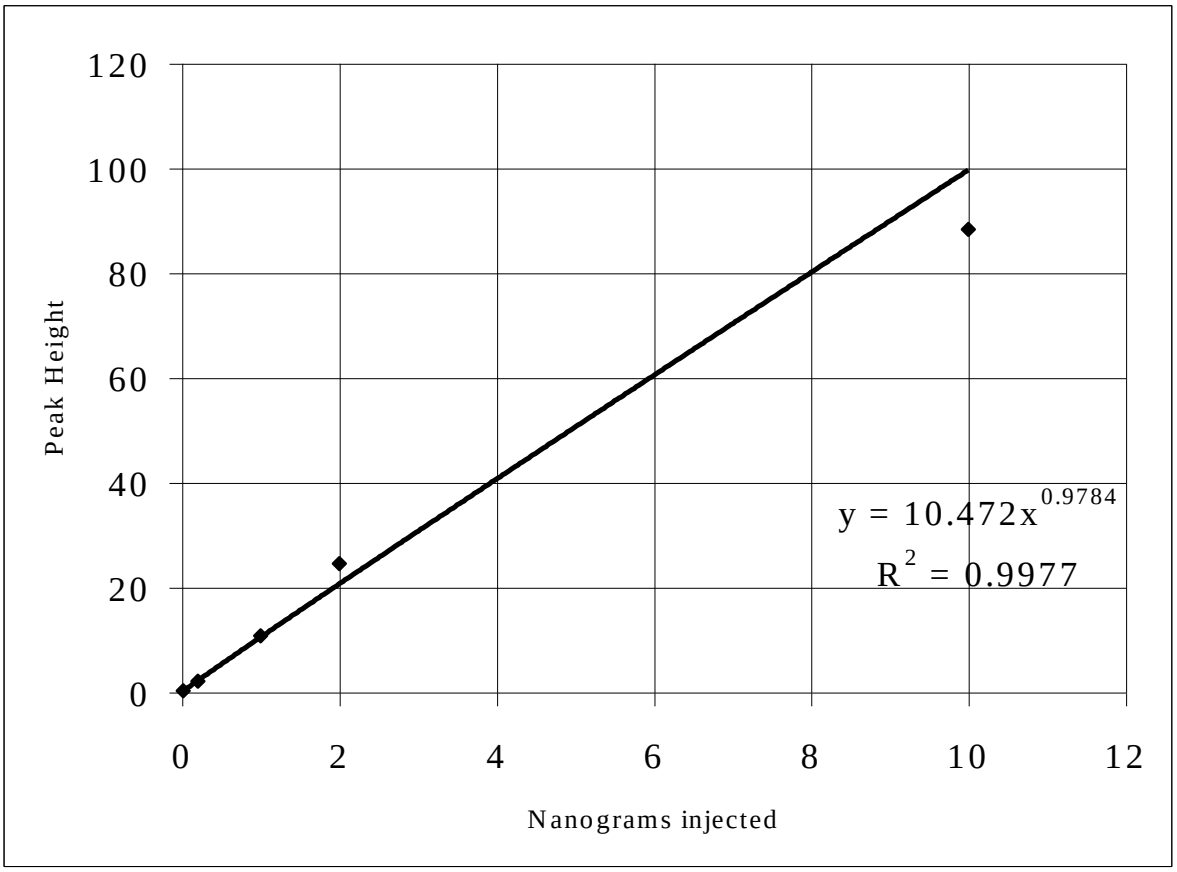


Fig 4.1: Karate Calibration Curve

The equation of the power fit curve for karate samples is $y=10.472x^{0.9784}$. This was used to calculate the concentrations of Karate in tea samples.

4.1.2 Pyagro calibration curve

Table 2: Pyagro calibration data

Nanograms injected	Peak height
0.02	1.5057
0.2	1.6805
1	4.2185
2	9.433
10	43.711

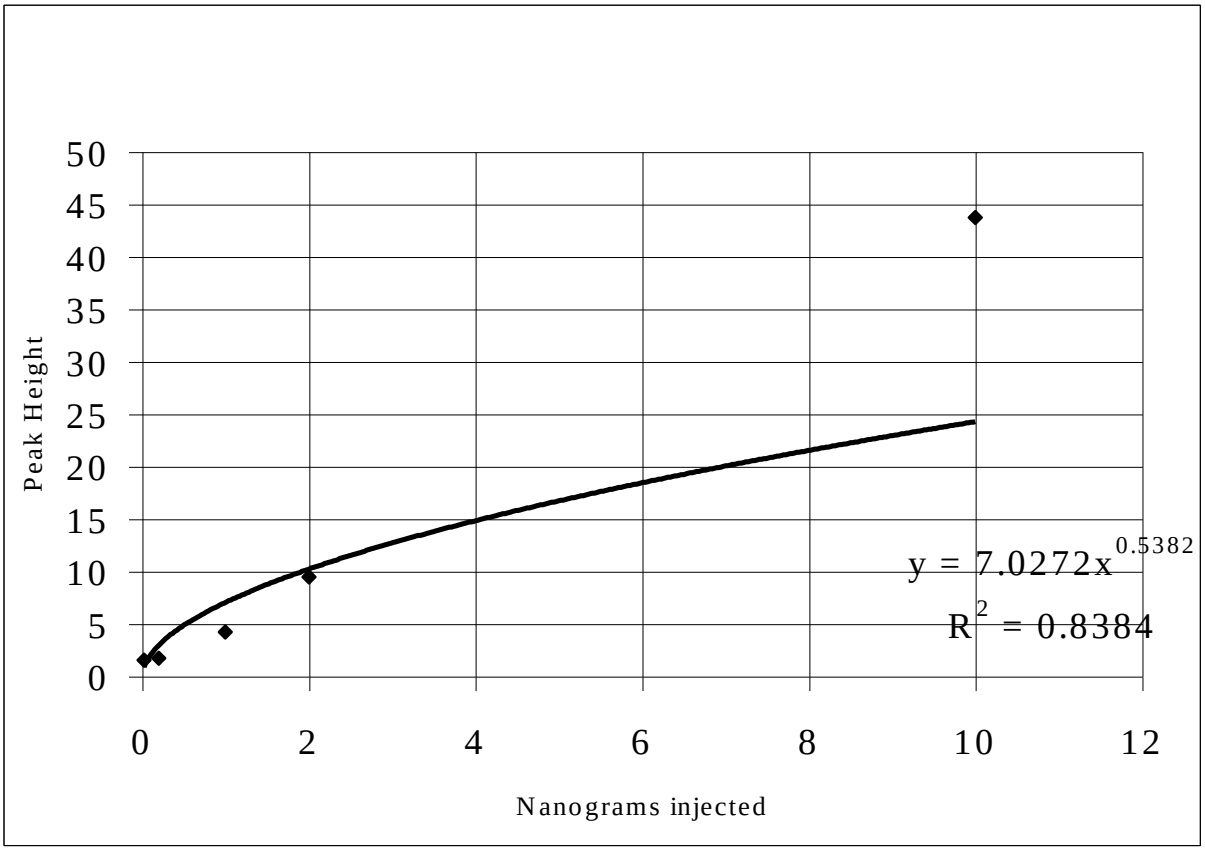


Fig 4.2: Pyagro Calibration Curve

The equation of the power fit curve for Pyagro samples is $y=7.0272x^{0.5382}$. This was used to calculate the concentrations of Pyagro in tea samples.

4.2 Calculation method

4.2.1 Sample concentration

The sample peak heights from chromatograms were used in the standard equation obtained to calculate the nanograms found for each sample. Some of the chromatograms for control samples, fortified samples and treated samples are given in appendix 2 to 18. The Pyrethrins and Lambda-cyhalothrin in all calculations were listed as ppm Pyagro and ppm karate respectively. The ppm of the pesticides was determined from the nanograms found from the calibration equation using the following steps:

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

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

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. The concentrations of all the samples analyzed are shown in the residue data sheets for Karate and Pyagro given as appendix 20 and 21 respectively. The mean residue in ppm for Karate and Pyagro are given in appendix 1.

4.2.2 Fortification recovery

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

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

The calculations of individual recoveries for this study are shown in appendix 19 and the summary of the percentage recoveries are as shown in the table below

Table 3: Fortification recovery for Karate and Pyagro

Pesticide	Sample type	% recovery
Karate	Fresh leaves	95.59
	Black tea	78.78
	Brewed tea	89.24
Pyagro	Fresh leaves	80.00
	Black tea	99.60
	Brewed tea	97.55

Treated sample residues were corrected upward using the percentage recoveries for each set of samples. The residues obtained after application of the correction factor have been referred to as corrected ppm and are shown in appendix 20 and 21.

4.3 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 Pyagro shown as figures 4.3, 4.4, 4.5, 4.6, 4.7 and 4.8 and the decay curves shown as figures; 4.9, 4.10, 4.11, 4.12 and 4.13 reveal that the pesticide residue levels decrease as the number of days after pesticide application increase. The data used to generate the decay curves for Karate and Pyagro are given in part (a) and (b) respectively of appendix 1.

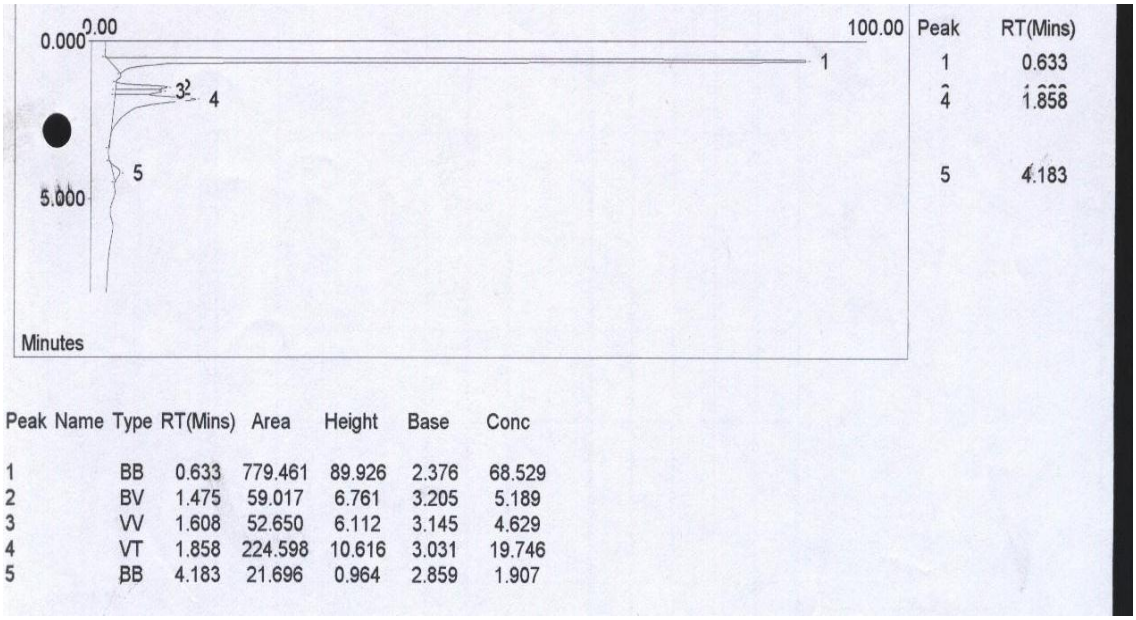
4.3.1 Chromatograms

4.3.1.1 Pyagro (Pyrethrins) chromatograms

The chromatograms given in figures 4.3, 4.4 and 4.5 show peaks for samples collected 0, 7 and 14-days after Pyagro pesticide application respectively. The figures indicate a fall in the pesticide concentration levels through the decrease in the peak height with time. Figure 4.3 shows five peaks three of which are Pyrethrins residue peaks. These are peak 3, 4 and 5. In

figure 4.4 one of the peaks present in figure 4.3 is absent leaving only two Pyrethrins peaks which are peak 3 and 4. In figure 4.5 only one Pyrethrin peak is remaining, which is peak 2. In addition to the disappearance of some peaks the heights of corresponding peaks is seen to gradually decrease. The residue levels recorded are based on the total peak heights of all the Pyrethrins peaks detected therefore decrease in the number of peaks and peak heights lead to decline in the pesticide residues. The decrease is caused by growth dilution (spread of the pesticide to new tissues as the plant grows, leading to lower concentrations as the number of tissues increase), photo degradation (i.e. Pyrethrins are decomposed by UV light) and rainfall which could wash away the pesticide from the leaf surface.

a) 0-day fresh leaf samples.



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BB	0.633	779.461	89.926	2.376	68.529
2		BV	1.475	59.017	6.761	3.205	5.189
3		VV	1.608	52.650	6.112	3.145	4.629
4		VT	1.858	224.598	10.616	3.031	19.746
5		BB	4.183	21.696	0.964	2.859	1.907

Fig 4.3: Pyagro 0-day fresh leaf sample chromatogram.

b) 7-day fresh leaf sample

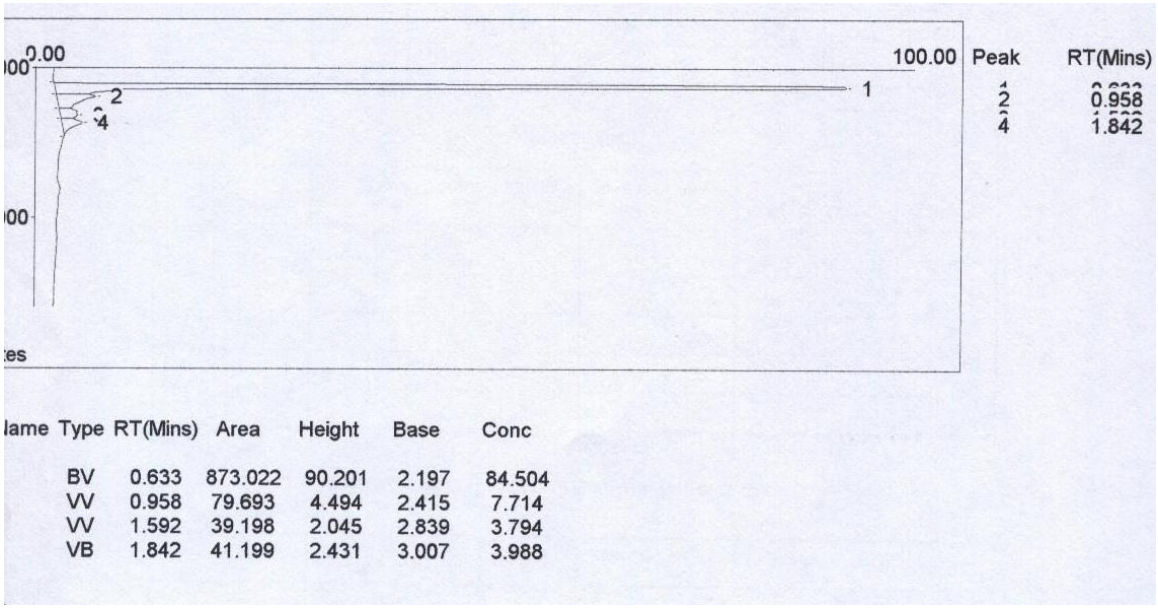


Fig 4.4: Pyagro 7-day fresh leaf sample chromatogram.

c) 14-day fresh leaf sample

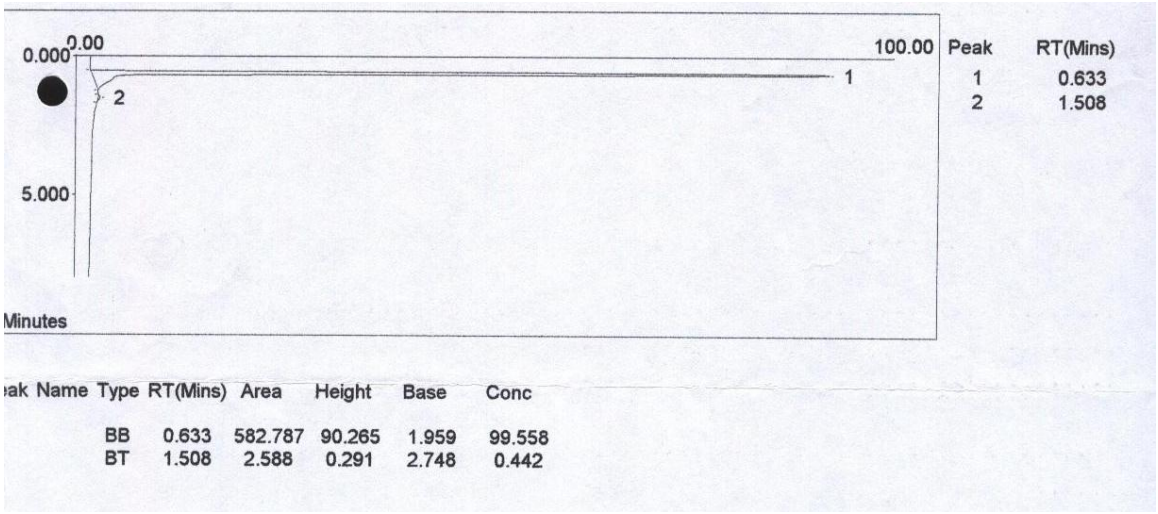
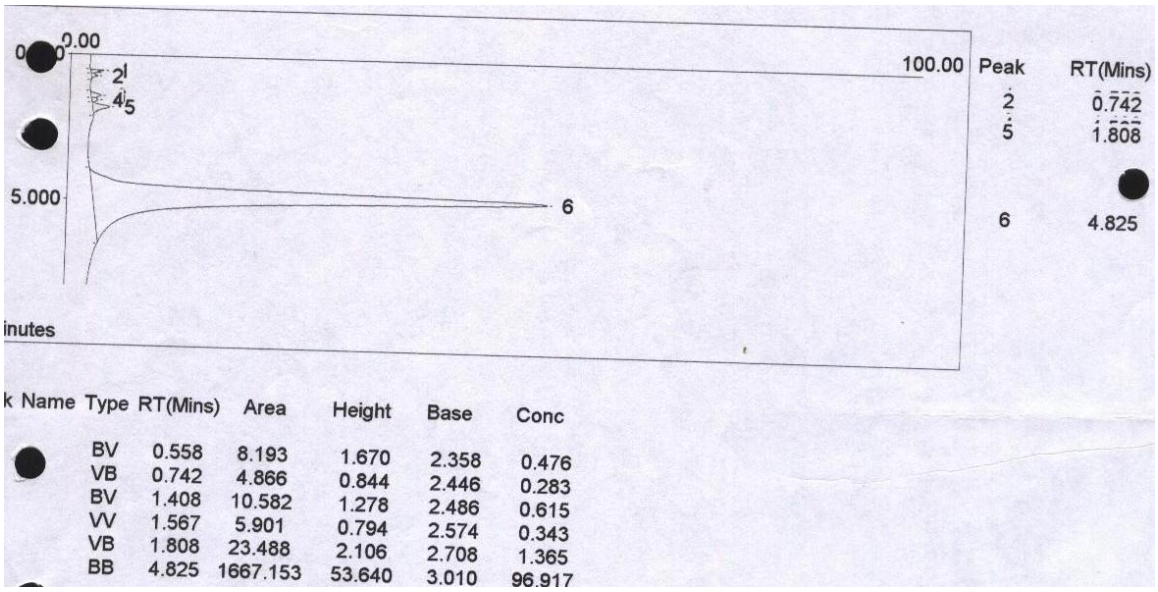


Fig 4.5: Pyagro 14-day fresh leaf sample chromatogram.

4.3.1.2 Karate (Lambda-cyhalothrin) chromatograms

The figures 8, 9 and 10 show chromatograms for samples collected on the 0, 7 and 14 days after Karate pesticide application respectively. The figures indicate a decline in the pesticide levels as the peak height decreases as the number of days after pesticide application increase. In figure 4.6, peak 6 is the peak of karate residues with a peak height of 53.640, the corresponding peak in figure 4.7, is peak 8 with peak height 4.024 and the corresponding peak in figure 4.8 is peak 7 with peak height 3.496. All these have a similar retention time of 4.8 minutes. Based on this observation the residue levels decrease as the number of days after application increase. The decrease is caused by growth dilution (spread of the pesticide to new tissues as the plant grows, leading to lower concentrations as the number of tissues increase), photo degradation (decomposition due to UV light) and rainfall which could wash away the pesticide from the leaf surface. (Muraleedharan *et, al* 2003)

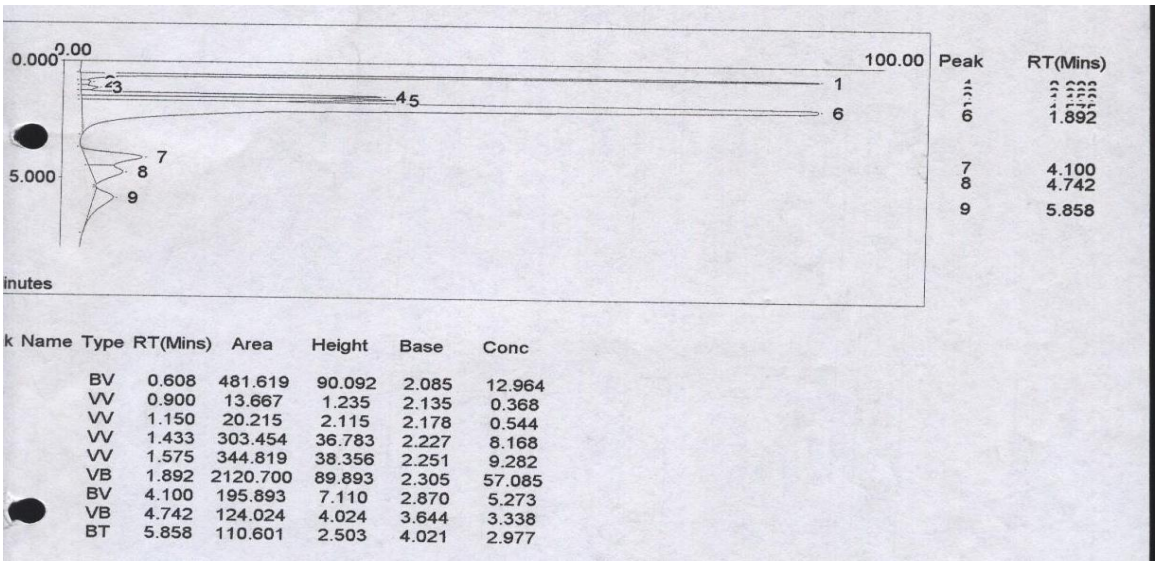
a) 0-day fresh leaf samples



k	Name	Type	RT(Mins)	Area	Height	Base	Conc
		BV	0.558	8.193	1.670	2.358	0.476
		VB	0.742	4.866	0.844	2.446	0.283
		BV	1.408	10.582	1.278	2.486	0.615
		VV	1.567	5.901	0.794	2.574	0.343
		VB	1.808	23.488	2.106	2.708	1.365
		BB	4.825	1667.153	53.640	3.010	96.917

Fig 4.6: Karate 0-day fresh leaf sample chromatogram.

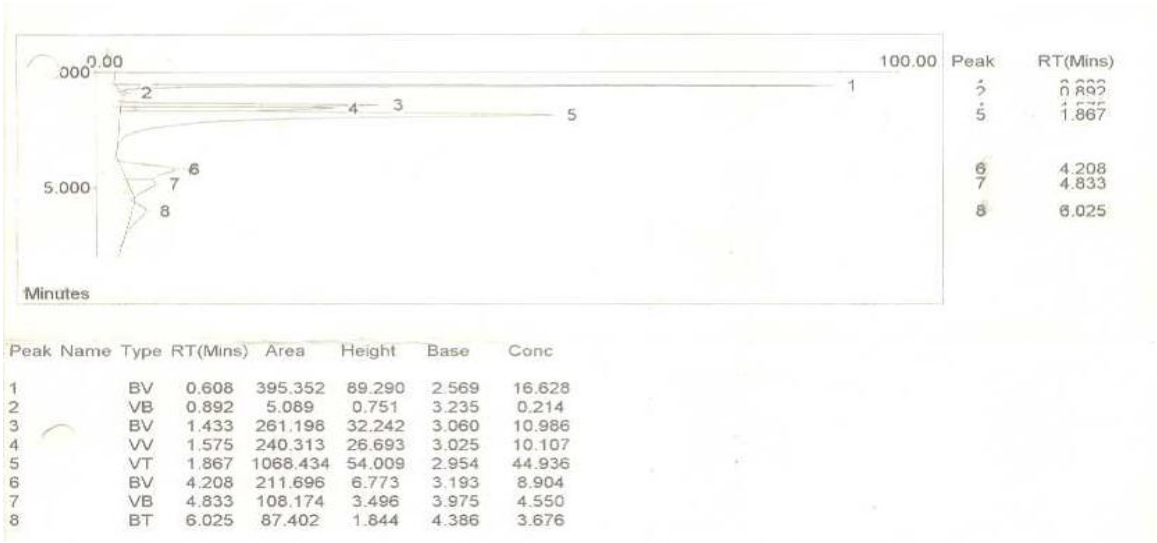
b) 7-day fresh leaf samples



k	Name	Type	RT(Mins)	Area	Height	Base	Conc
		BV	0.608	481.619	90.092	2.085	12.964
		VV	0.900	13.667	1.235	2.135	0.368
		VV	1.150	20.215	2.115	2.178	0.544
		VV	1.433	303.454	36.783	2.227	8.168
		VV	1.575	344.819	38.356	2.251	9.282
		VB	1.892	2120.700	89.893	2.305	57.085
		BV	4.100	195.893	7.110	2.870	5.273
		VB	4.742	124.024	4.024	3.644	3.338
		BT	5.858	110.601	2.503	4.021	2.977

Fig 4.7**:** Karate 7-day fresh leaf sample, chromatogram.

c) 14-day fresh leaf sample



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BV	0.608	395.352	89.290	2.569	16.628
2		VB	0.892	5.089	0.751	3.235	0.214
3		BV	1.433	261.196	32.242	3.060	10.986
4		VV	1.575	240.313	26.693	3.025	10.107
5		VT	1.867	1068.434	54.009	2.954	44.936
6		BV	4.208	211.696	6.773	3.193	8.904
7		VB	4.833	108.174	3.496	3.975	4.550
8		BT	6.025	87.402	1.844	4.386	3.676

Fig 4.8: Karate 14-day fresh leaf sample, chromatogram.

4.3.2 Residue decay curves

4.3.2.1 Karate residue decay curves

The residue decay curves given in figures 4.9 and 4.10 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. Figure 4.9 shows decay in the fresh leaves, this decay is mainly attributed to growth dilution, photo degradation and rainfall. Figure 4.10 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.

a) Fresh leaf samples

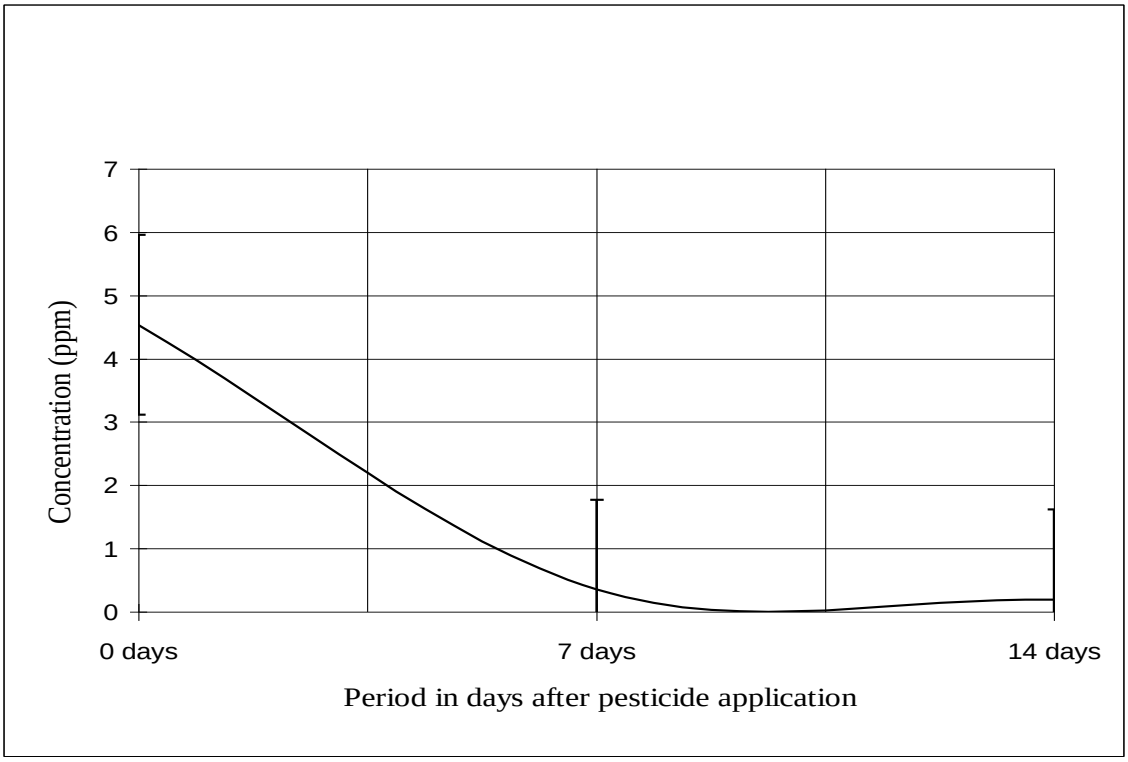


Fig 4.9: Karate fresh leaf samples decay curve

b) Black tea samples

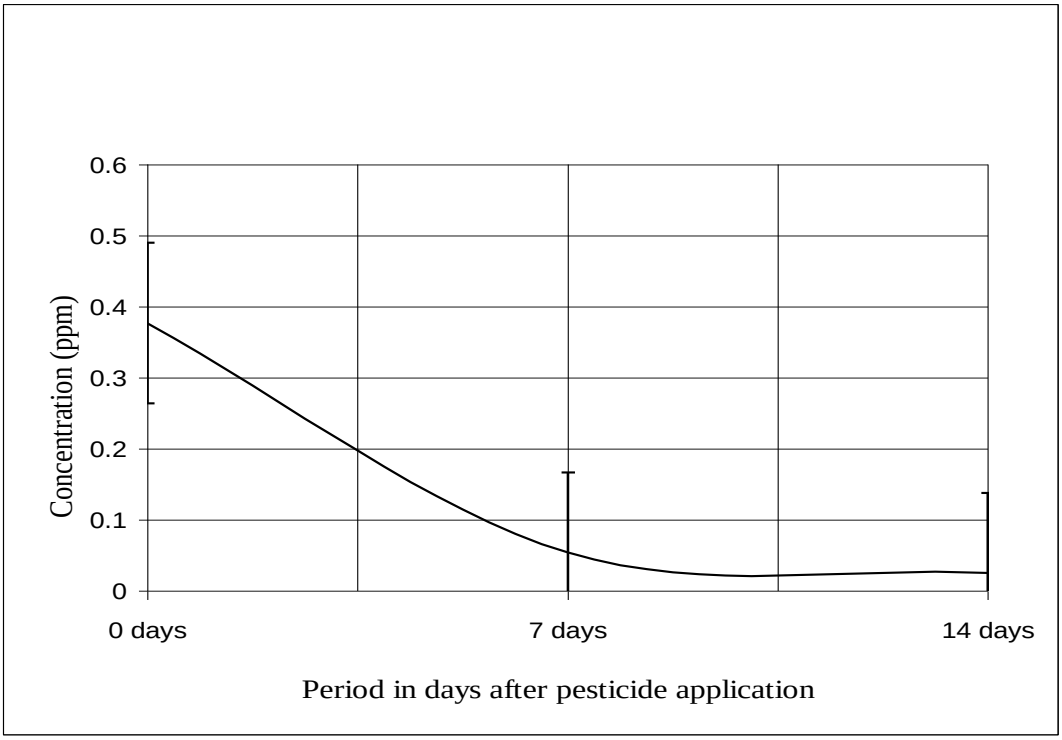


Fig 4.10: Karate Black tea samples decay curve

4.3.2.2 Pyagro pesticide residue decay curves

The residue decay curves given in figures 4.11, 4.12 and 4.13 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 and also as they undergo tea preparation procedures. Figure 4.11 shows decay in the fresh leaves, this decay is mainly attributed to growth dilution, photo degradation and rainfall. Figure 4.12 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 manufacture of black tea. Figure 4.13 shows residues in brewed tea which are further reduced by high temperatures during brewing. The decay curves for the fresh tea keaf samples go below zero because of the big range between the values for zero day sample (1.077 ppm) and the 14-day value (0.001). Similarly the curve for the black tea sample due the range between the values for zero day samples (0.160 ppm) and the 14-day value (0.0003 ppm)

a) Fresh leaf samples

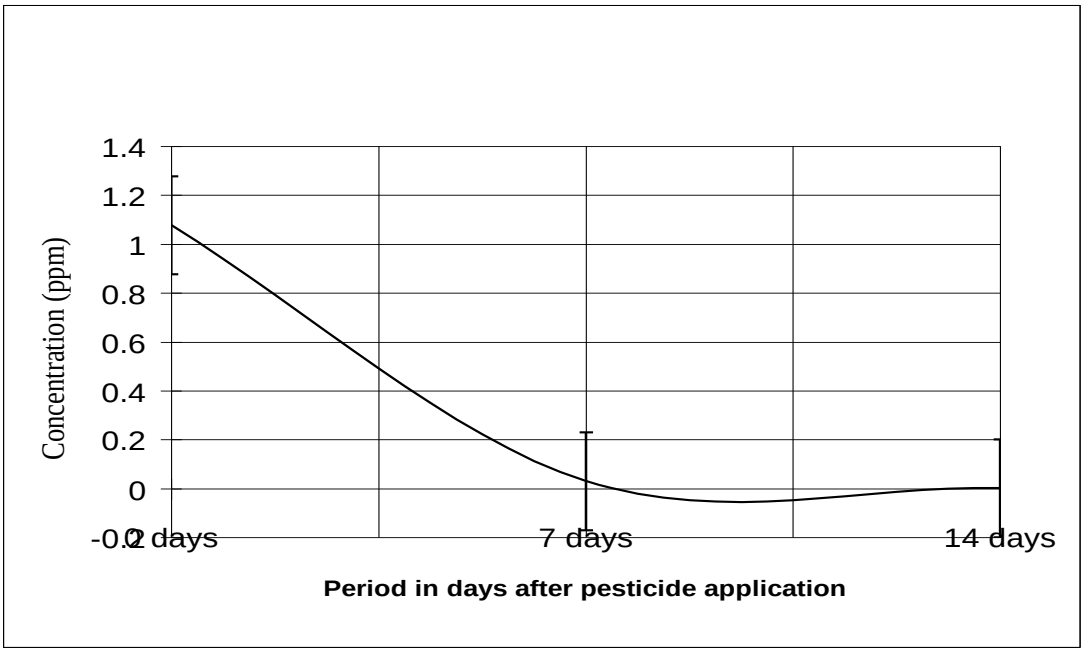


Fig 4.11: Pyagro fresh leaf samples decay curve

b) Black tea samples

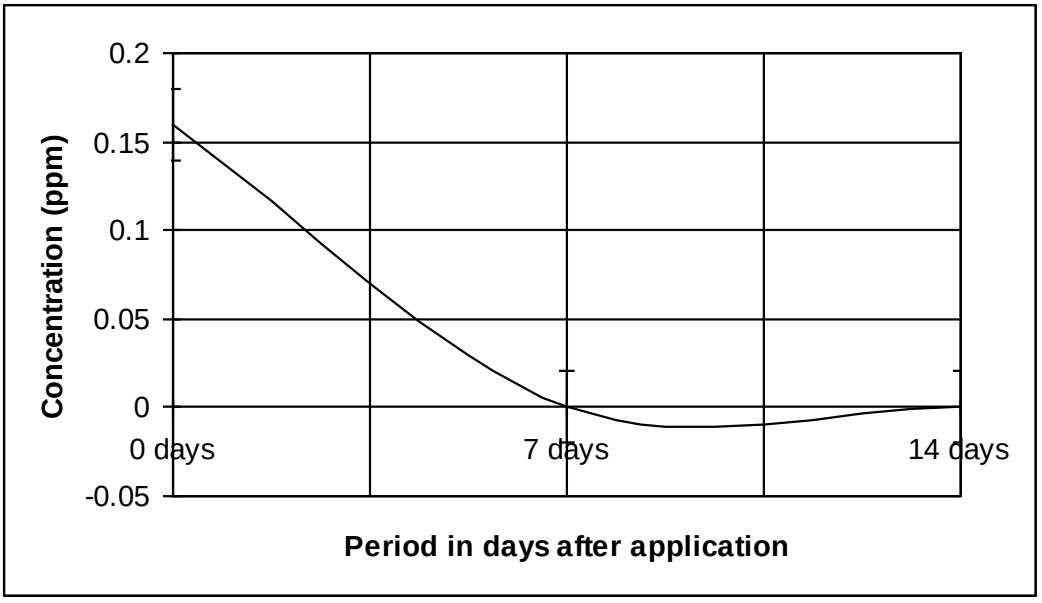


Fig 4.12: Pyagro Black tea samples decay curve

c) Brewed tea samples

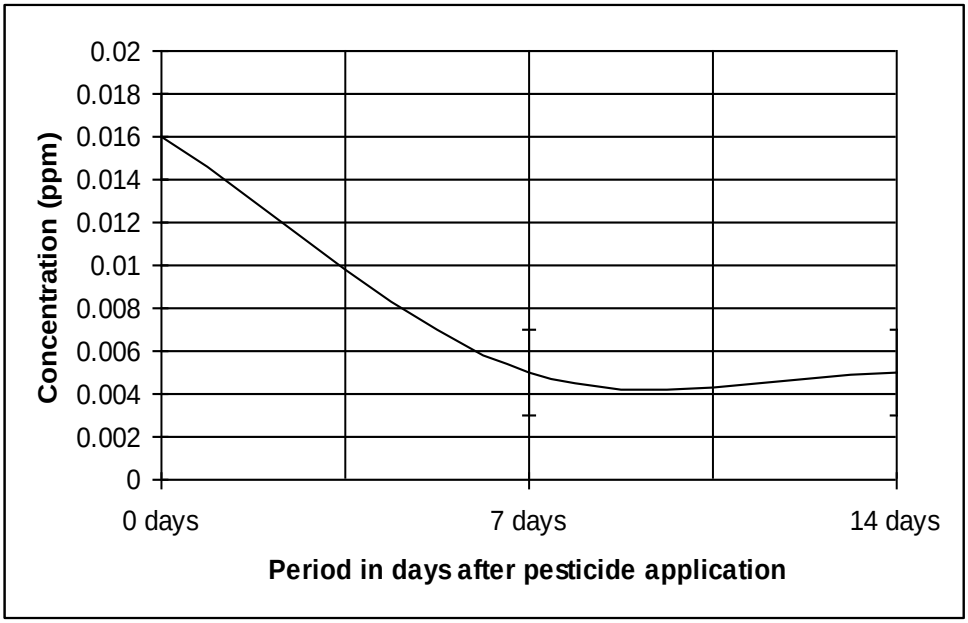


Fig 4.13: Pyagro Brewed tea samples decay curve

4.4 Effect of processing on pesticide residue levels

Pesticides are generally lost before the manufacture of black tea, by evaporation, rainfall, photo degradation and through growth dilution. Further degradation 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 4.14, 4.15, 4.16, 4.17 and 4.18. Pesticide residues may leach into the brewed tea or remain in the spent tea depending on their solubility in water. Figure 4.14, 4.15 and 4.16 show that there are no karate residues remaining in the brewed tea. On the other hand, Pyagro residues pass into the brewed tea though in small amounts as shown in figure 4.17 and 4.18

4.4.1 Karate samples effect of processing on pesticide residue levels

a) 0-day Samples

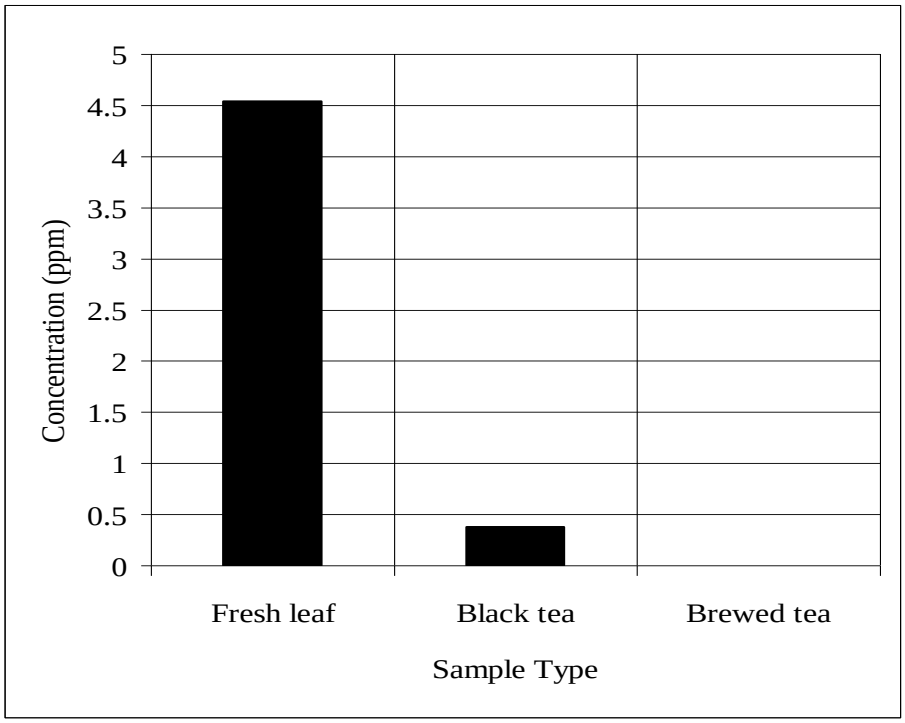


Fig 4.14: Karate 0-day; Effect of processing on residue levels.

Figure 4.14 above show a decline of pesticide residues as black tea is processed from the fresh leaf and as brewed tea is made from black tea. The samples were collected on the day application of karate was carried out. There pesticide residues decline from 4.535 ppm in fresh leaf sample to 0.377 ppm in black tea as shown in appendix 1. This is a percentage decrease of 91.7%. This decline can be attributed to thermal decomposition due to high temperatures of about 120°C during the manufacture of black tea. There were no detectable residues in the brewed tea; this could be due to insolubility of lambda-cyhalothrin water or thermal decomposition and eventual evaporation during brewing.

b) 7-day samples

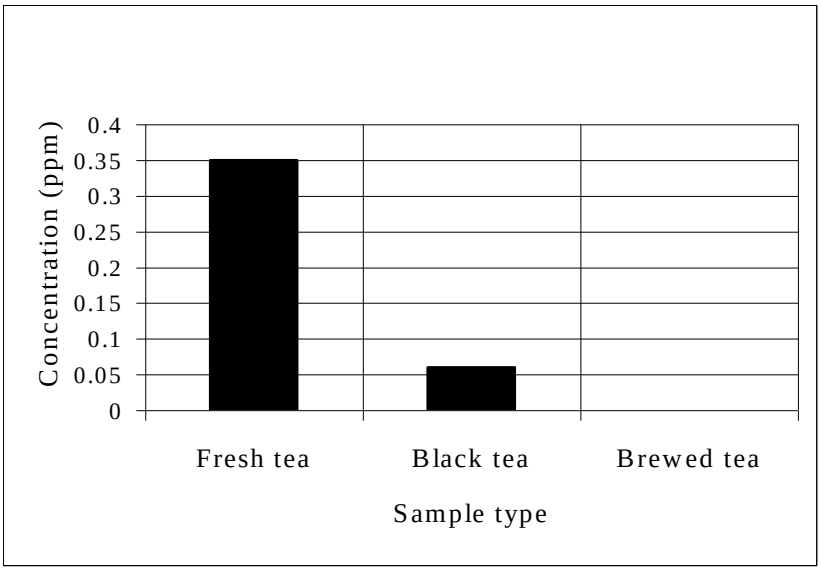


Fig 4.15: Karate 7-day; Effect of processing on residue levels.

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) and to undetectable levels in the brewed tea for samples collected 7-days after pesticide application. These could be attributed to thermal decomposition and insolubility in water respectively.

c) 14- day samples

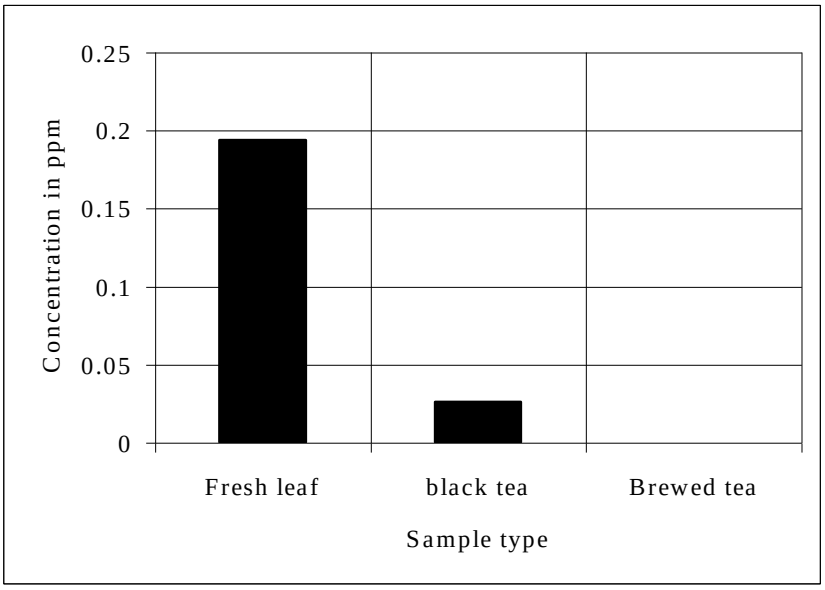


Fig 4.16: Karate 14-day; Effect of processing on residue levels.

The bar graph shows a steady decline of the already low lambda-cyhalothrin 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 is mainly due to thermal decomposition as a result of high temperatures during processing. 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.

4.4.2 Pyagro samples on effect of processing on pesticide residue levels

a) 0-day Samples

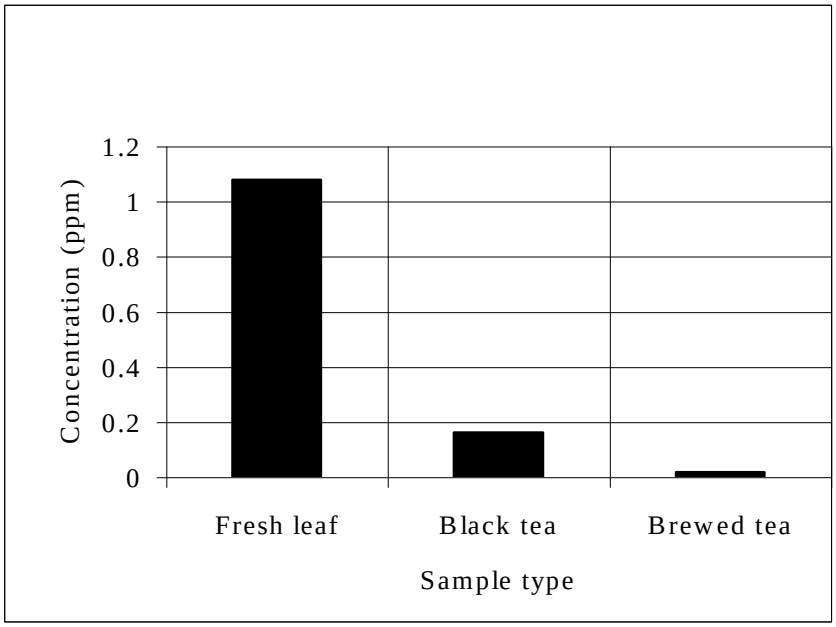


Fig 4.17: Pyagro 0-day; Effect of processing on residue levels.

Figure 4.17 shows a decrease in Pyrethrins residues in the different tea matrices, which were collected on the first day after spraying tea with Pyagro. There is a clear drop from 1.077 ppm in the fresh leaf sample to 0.16 ppm in the black tea, a decrease of 85.1%. This decrease could be as a result of exposure to high temperatures of about 120°C during manufacture of black tea. The graph shows a decrease of 90% from 0.16 ppm in black tea to 0.016 ppm in the brewed tea, this suggests that Pyrethrins are soluble in water and leach into the tea infusion. The residue level in the brewed tea is much lower than the residues found in the black tea; this could be attributed to exposure to high temperatures during brewing. The high temperatures cause decomposition of the residues as Pyagro is said to degrade at temperatures above 70^0C (Wangai and Kitazi, 2001).

b) 7-day samples

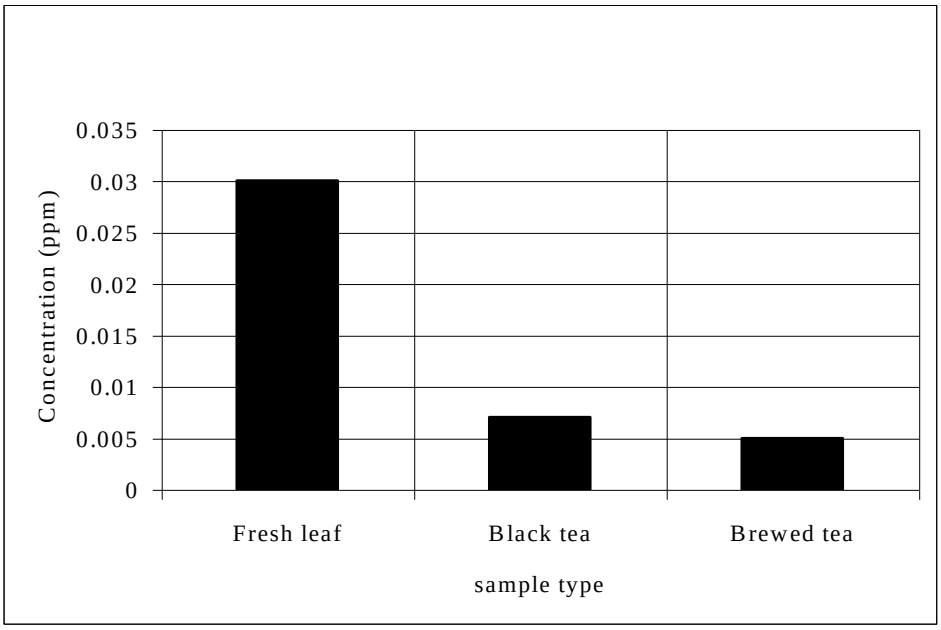


Fig 4.18: Pyagro 7-day; Effect of processing on residue levels.

The residue levels found in the 7- day samples are lower than the residue levels found in the 0- day samples. Figure 20 shows a steady decrease from 0.03 ppm in the fresh leaf sample to 0.007 ppm in the black tea, a 76.7% decrease. This decrease could be due to exposure to high temperatures of about 120°C during the manufacture of black tea. Brewed tea contains 0.005 ppm which is lower than the concentration in the black tea by 28.6%. The decrease could be due to exposure to high temperatures during brewing of tea. The high temperatures may have caused decomposition of the residues and subsequent evaporation. (Wangai and Kitazi, 2001).

c) 14- day samples

The residues found in the tea matrices collected on the 14th day after pesticide application were very low thus the difference between the residues in the three matrices may be insignificantly different. The residues are much lower than the residues detected in the 7th day samples. The residues shown in table V in appendix 1, decrease from 0.0007 ppm in the fresh

leaf tea matrix to 0.0003 ppm in black tea. This decline could be due to thermal decomposition and subsequent evaporation during manufacture of black tea. The residue found in the brewed tea was found to be 0.0005 ppm, which is higher than the residues in the black tea, this could be due to the low concentrations hence the margin of experimental error could make the values overlap.

The graph for the residue values obtained from the 14-day samples may not give the actual effect of processing on residue level therefore it is not presented.

CHAPTER FIVE

CONCLUSION AND RECOMMENTATIONS

5.1 Conclusion

The mean residue data in appendix 1, the chromatograms, residue decay curves and the bar graphs show that application of Karate and Pyagro 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 pesticides at maximum proposed rates indicate that the correct time of harvesting (pre-harvest interval) after pesticide application is seven days. It was also established that tea processing and preparation play an important role in reducing the pesticide residue levels.

The trial site was located in Kericho, one of the most productive areas in Kenya, where the conditions are appropriate for tea cultivation. The trial site was representative of diverse tea growing areas of western Kenya. Karate 1.75E.C and Pyagro 4.0E.C are 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 maximum recommended rate for Karate 1.75E.C is 3ml/liter of water and for Pyagro 4.0 E.C is 5ml/liter of water. The application parameters considered during the field trial may be proposed for use in Kericho

The stability of the analyte in frozen storage within tea matrices has been substantiated by past storage experiments which concluded that Lambda-cyhalothrin is stable in frozen crop samples for several months. (FAO, 1986). The results are therefore supported in terms of matrix stability in storage prior to analysis. More so, Pyrethrins are said to be stable under frozen conditions. (Wangai *et al* 2001)

The methods used in this study were based on well-established procedures for lambda-cyhalothrin and Pyrethrin analysis in a wide range of matrices. Fresh tea leaves, black tea and brewed tea were analyzed. Samples analyzed included samples fortified at two levels, 1x and 100x the method limit of quantitation (LOQ). These levels do not include residues in all samples generated according to the proposed use.

Karate residues in the various tea matrices were higher than the method LOQ of 0.01ppm, 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. Pyagro residues were lower than the method LOQ of 0.05ppm except for the fresh tea and black tea samples taken on the day of pesticide application.

Based on the results presented above; treatment of mature tea bushes with Pyagro 4EC and Karate 1.75EC according to the intended use pattern tested in Kericho will produce very low residue levels of pyrethrin (pyagro) residues in brewed tea and no detectable residue levels of lambda-cyhalothrin (Karate) in brewed tea. Generally it will give low levels of both pyrethrin and lambda-cyhalothrin 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 these pesticides if used according to the intended instructions will pose no risks to the consumers of tea. More so, the methods of tea preparation used in brewing tea in this study should be considered a worst case procedure used by the consumer. This is because in most cases tea is prepared by boiling water with black tea together hence exposing the residues to higher temperatures which cause their decomposition, thus the residues passed on to the consumer are presumed to be less than the acceptable daily intake (ADI) set by the various international regulatory bodies.

The pesticides residues in tea are generally lost from the fresh leaves by evaporation, growth dilution (spread of pesticide to new tissues resulting from plant growth lowering pesticide concentration per plant cell), photo degradation and rainfall. Further degradation occurs due to thermal decomposition and evaporation during the manufacture of black tea and during brewing when tea is exposed to high temperatures. (Muraleedhan *et al*, 2003)

5.2 Recommendations

From the results of the study the following recommendations could be made on the residues found in tea after application of Karate and Pyagro.

1) Tea farmers in Kenya should be advised to use Karate and Pyagro pesticides because their residues have been established and when used according to Good Agricultural Practice (GAP), will not result in unacceptable residues in the tea product.
2) When using the two chemicals (Karate and Pyagro) farmers should follow the stipulated recommendations on the dosage and dilution rates for each pesticide as shown on the

label. They should also follow all the safety measures including wearing protective clothes and time of application.

3) The results indicate that 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. Plucking of tea leaves should not be done before a period of seven days after spraying with the two pesticides.

4) The pesticide residues found in dried (black) tea are below those of international standards (0.1ppm) therefore their use do not pose any risk to consumers of tea.

5) A study of the residues of Karate and Pyagro in tea in other tea growing regions in Kenya could be of great importance to be able to determine the residues under different environmental conditions. Studies of the residues of the two pesticides in other types of tea such as instant tea and green tea will be important to cater for consumers of this tea types.

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APPENDICES

Appendix 1

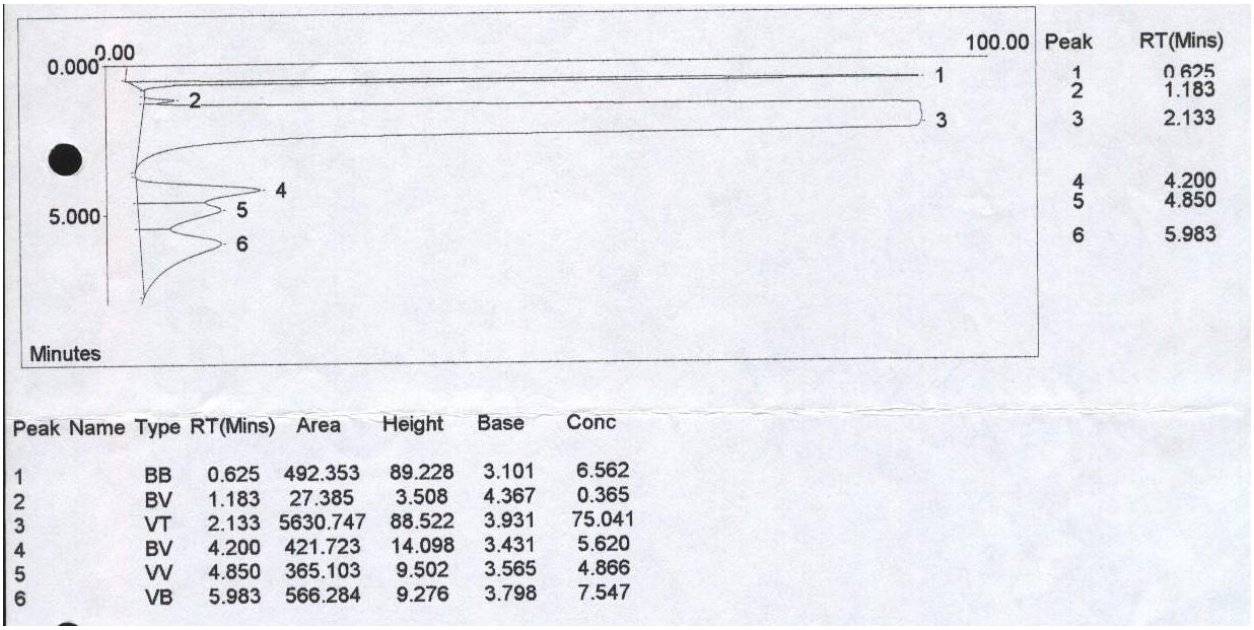
a) Summary of Karate residue (mean ppm) in the different tea matrices sampled at different intervals after pesticide application.

Sample type	Period in days after pesticide application: 0 days	7 days	14 days
Fresh leaf	4.535	0.349	0.194
Black tea	0.377	0.059	0.026
Brewed tea	0	0	0

b) Summary of Pyagro residue (mean ppm) in the different tea matrices sampled at different intervals after pesticide application.

Sample type	Period in days after pesticide application: 0 days	7 days	14 days
Fresh leaf	1.077	0.03	0.0007
Black tea	0.16	0.007	0.0003
Brewed tea	0.016	0.005	0.0005

Appendix 2



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BB	0.625	492.353	89.228	3.101	6.562
2		BV	1.183	27.385	3.508	4.367	0.365
3		VT	2.133	5630.747	88.522	3.931	75.041
4		BV	4.200	421.723	14.098	3.431	5.620
5		VV	4.850	365.103	9.502	3.565	4.866
6		VB	5.983	566.284	9.276	3.798	7.547

Fresh leaves fortified sample

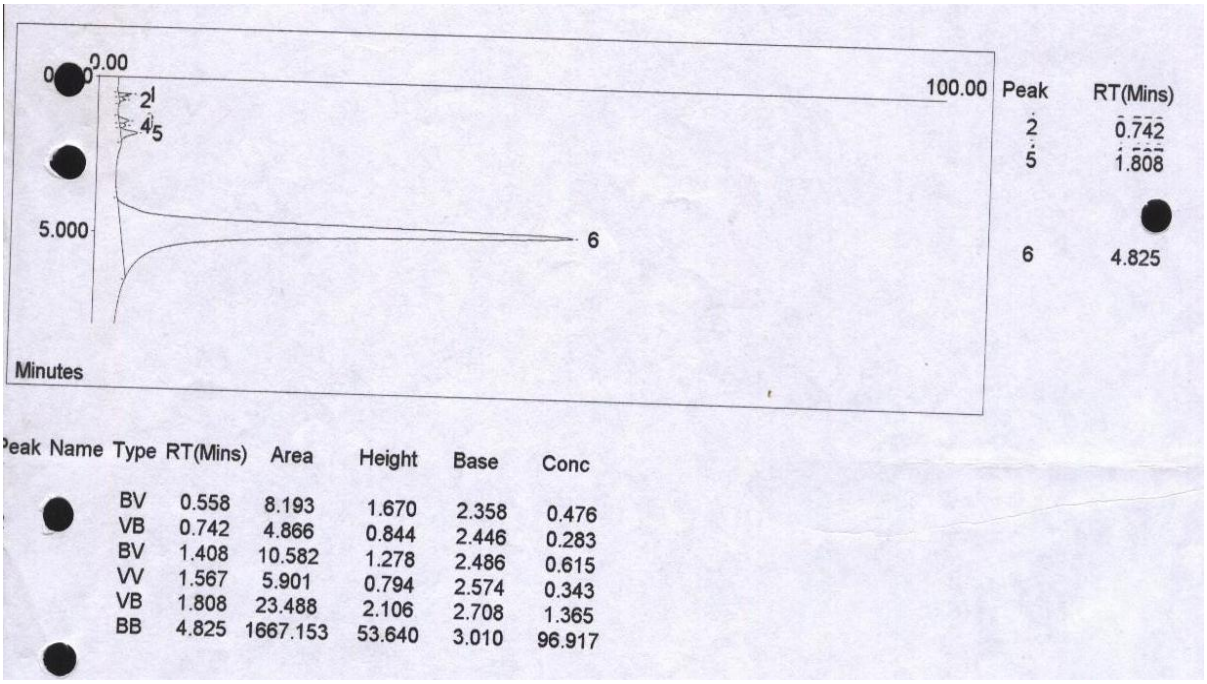
Sample identification: Karate KF-2

Sample number: S_{12}

Analysis date: 21st April 2004

Amount found: 0.9557 (95.57% recovery)

Appendix 3



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
		BV	0.558	8.193	1.670	2.358	0.476
		VB	0.742	4.866	0.844	2.446	0.283
		BV	1.408	10.582	1.278	2.486	0.615
		VV	1.567	5.901	0.794	2.574	0.343
		VB	1.808	23.488	2.106	2.708	1.365
		BB	4.825	1667.153	53.640	3.010	96.917

Fresh leaves treated sample

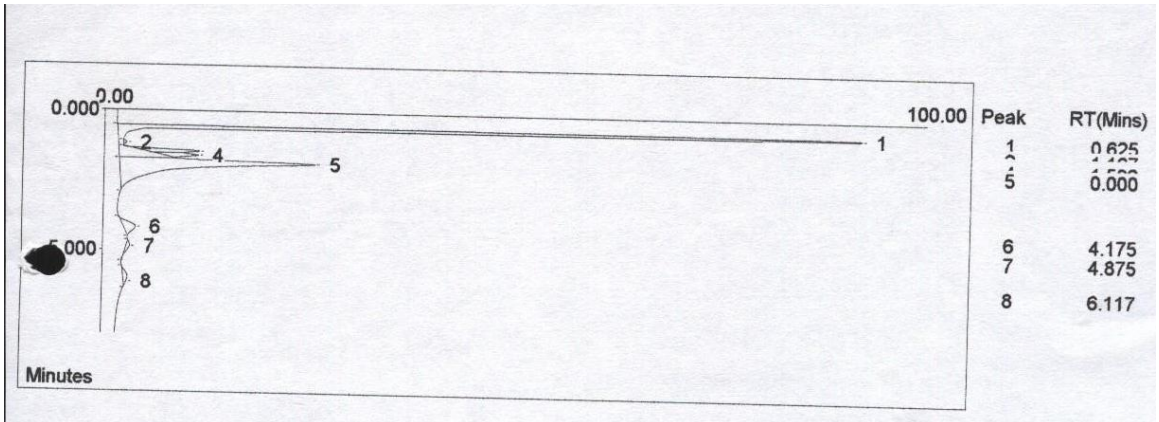
Sample identification: Karate 1.3 0day

Sample number: S_2

Analysis date: 7^{nth}April 2004

Amount found: 4.55 (4.766ppm corrected)

Appendix 4



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BBE	0.625	644.888	90.678	1.554	81.847
2		VVS	1.167	2.431	0.380	2.369	0.309
3		VVS	1.442	48.899	6.799	4.889	6.206
4		VVS	1.592	31.569	4.403	7.158	4.007
5		VB	0.000	0.000	23.966	1.859	0.000
6		BB	4.175	33.154	1.526	2.508	4.208
7		BT	4.875	10.773	0.495	2.846	1.367
8		BB	6.117	16.205	0.415	2.603	2.057

Black tea control sample

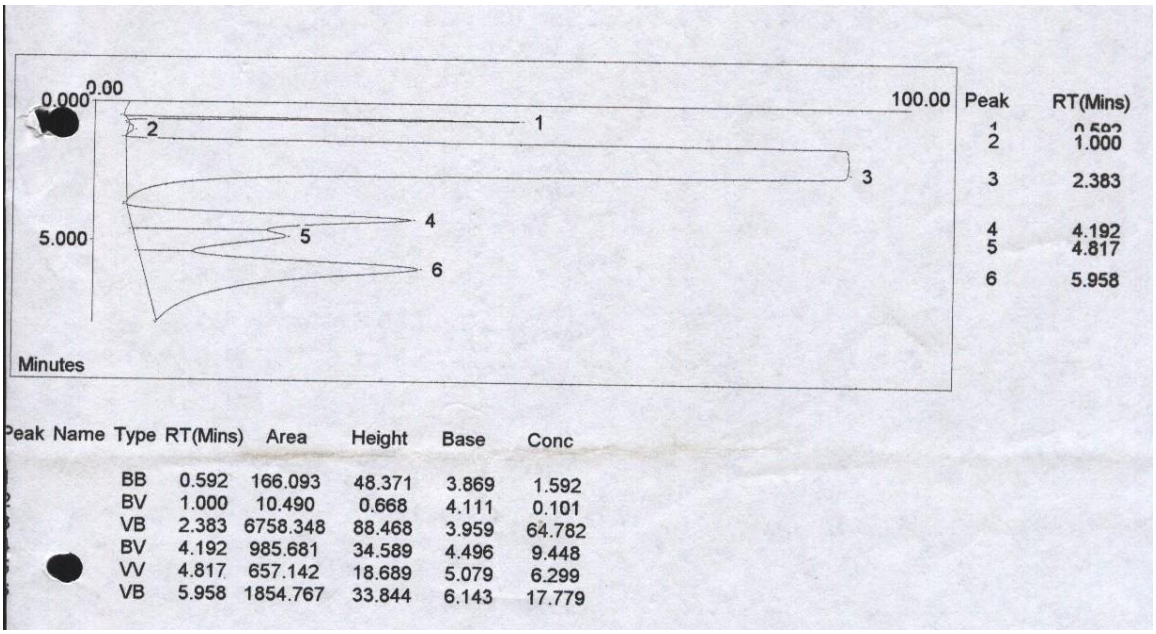
Sample identification: Karate MFK control

Sample number: S_{16}

Analysis date: 23rd April 2004

Amount found: 0.027ppm

Appendix 5



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
		BB	0.592	166.093	48.371	3.869	1.592
		BV	1.000	10.490	0.668	4.111	0.101
		VB	2.383	6758.348	88.468	3.959	64.782
		BV	4.192	985.681	34.589	4.496	9.448
		VV	4.817	657.142	18.689	5.079	6.299
		VB	5.958	1854.767	33.844	6.143	17.779

Black tea fortified sample

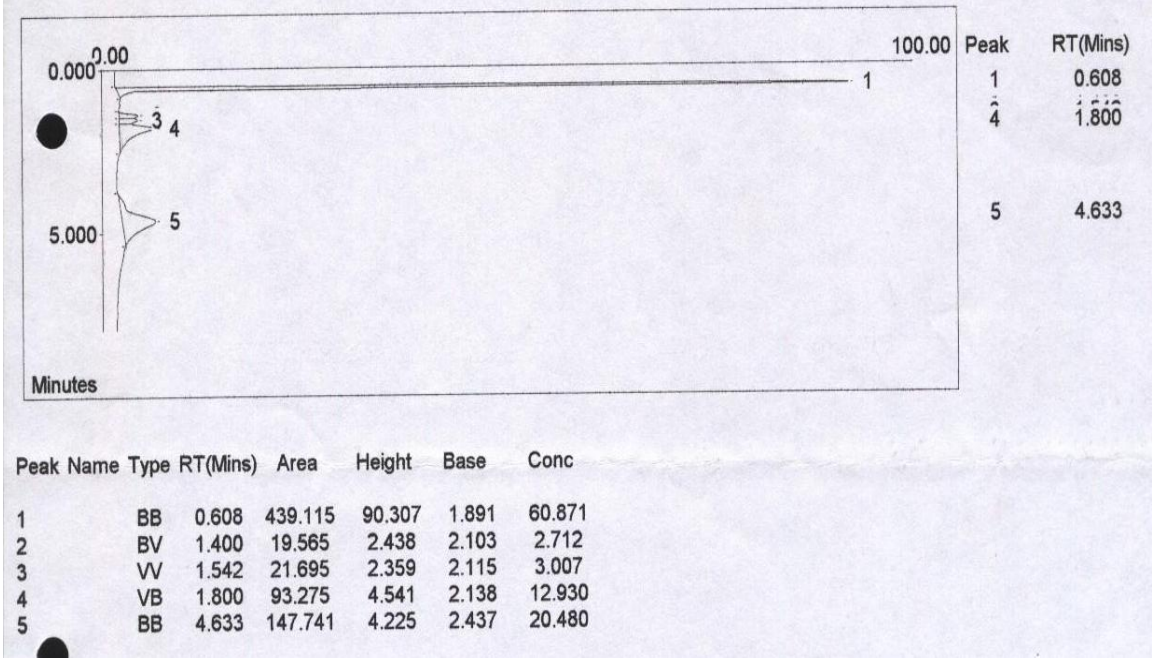
Sample identification: Karate MFKF-2

Sample number: S_{24}

Analysis date: 27^{nth} April 2004

Amount found: 0.8925ppm (86.55% recovery)

Appendix 6



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BB	0.608	439.115	90.307	1.891	60.871
2		BV	1.400	19.565	2.438	2.103	2.712
3		VV	1.542	21.695	2.359	2.115	3.007
4		VB	1.800	93.275	4.541	2.138	12.930
5		BB	4.633	147.741	4.225	2.437	20.480

Black tea treated sample

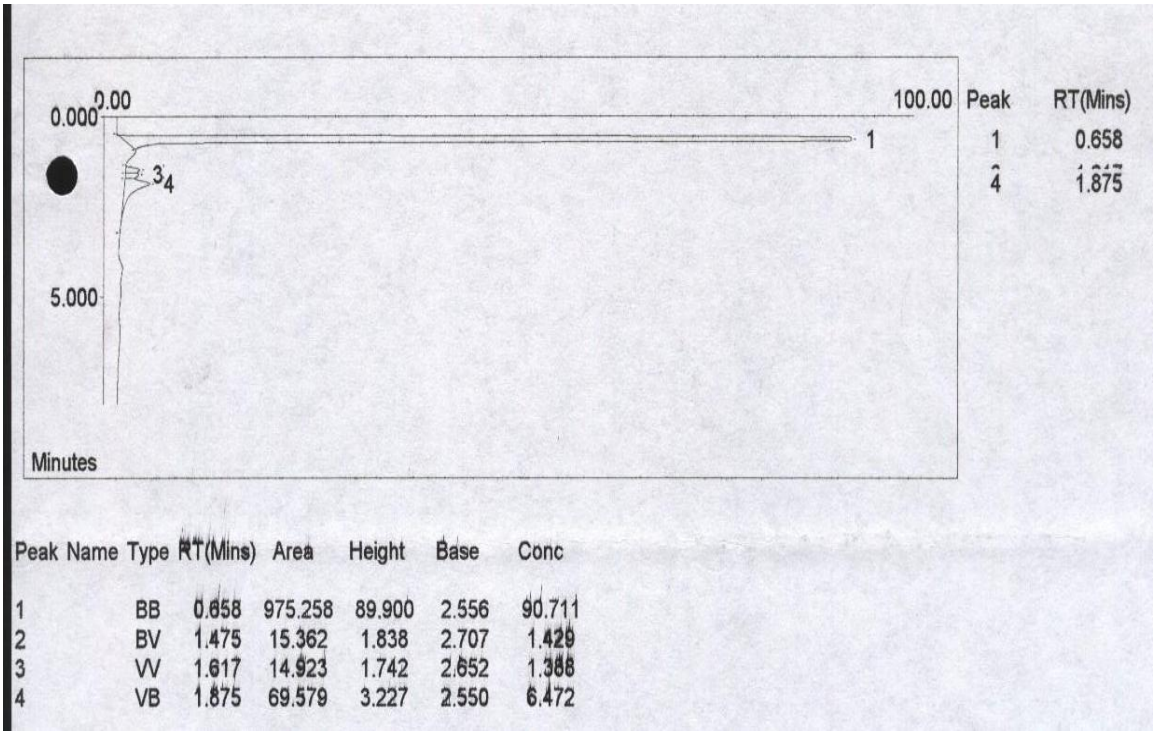
Sample identification: Karate MFK-3, 0 day

Sample number: S_{15}

Analysis date: 22nd April 2004

Amount found: 0.4158ppm (0.55277ppm)

Appendix 7



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BB	0.658	975.258	89.900	2.556	90.711
2		BV	1.475	15.362	1.838	2.707	1.429
3		VV	1.617	14.923	1.742	2.652	1.388
4		VB	1.875	69.579	3.227	2.550	6.472

Brewed tea control sample

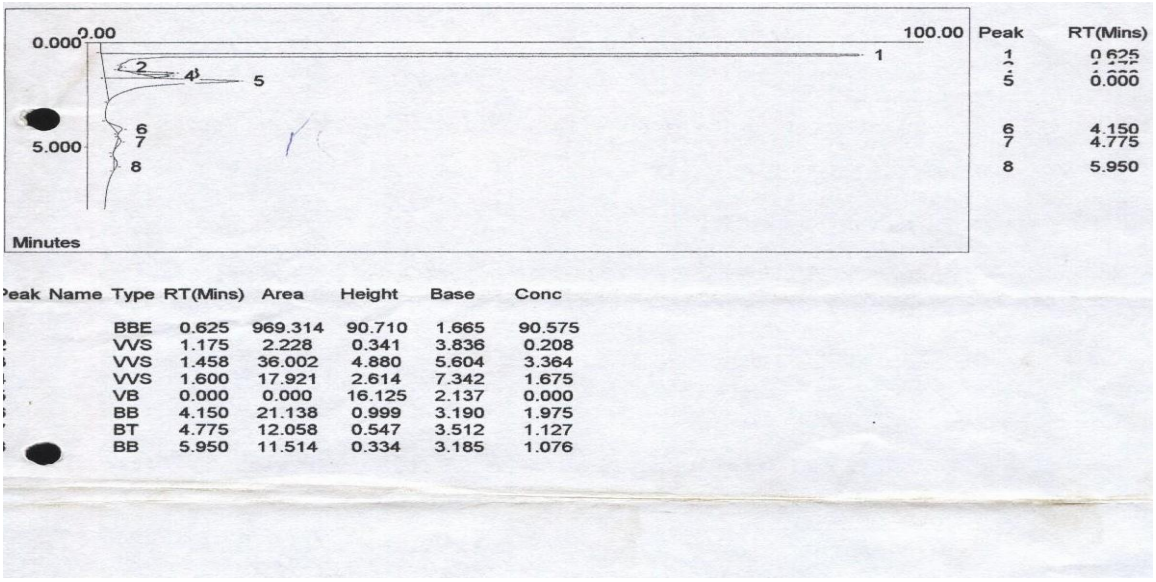
Sample identification: Karate MFKB- control

Sample number: S_{25}

Analysis date: 28th April 2004

Amount found: ND

Appendix 8



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
		BBE	0.625	969.314	90.710	1.665	90.575
		VVS	1.175	2.228	0.341	3.836	0.208
		VVS	1.458	36.002	4.880	5.604	3.364
		VVS	1.600	17.921	2.614	7.342	1.675
		VB	0.000	0.000	16.125	2.137	0.000
		BB	4.150	21.138	0.999	3.190	1.975
		BT	4.775	12.058	0.547	3.512	1.127
		BB	5.950	11.514	0.334	3.185	1.076

Brewed tea fortified sample

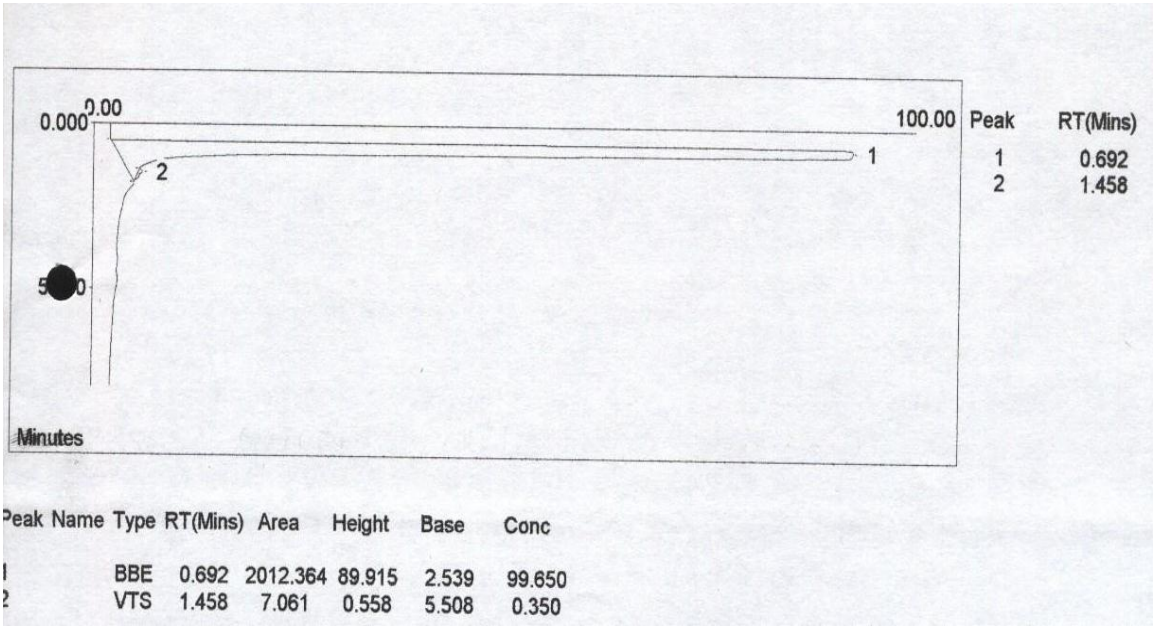
Sample identification: Karate MFKBF-1, 0 day

Sample number: S_{35}

Analysis date: 30th April 2004

Amount found: 0.008167 (81.67% recovery)

Appendix 9



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BBE	0.692	2012.364	89.915	2.539	99.650
2		VTS	1.458	7.061	0.558	5.508	0.350

Brewed tea treated sample

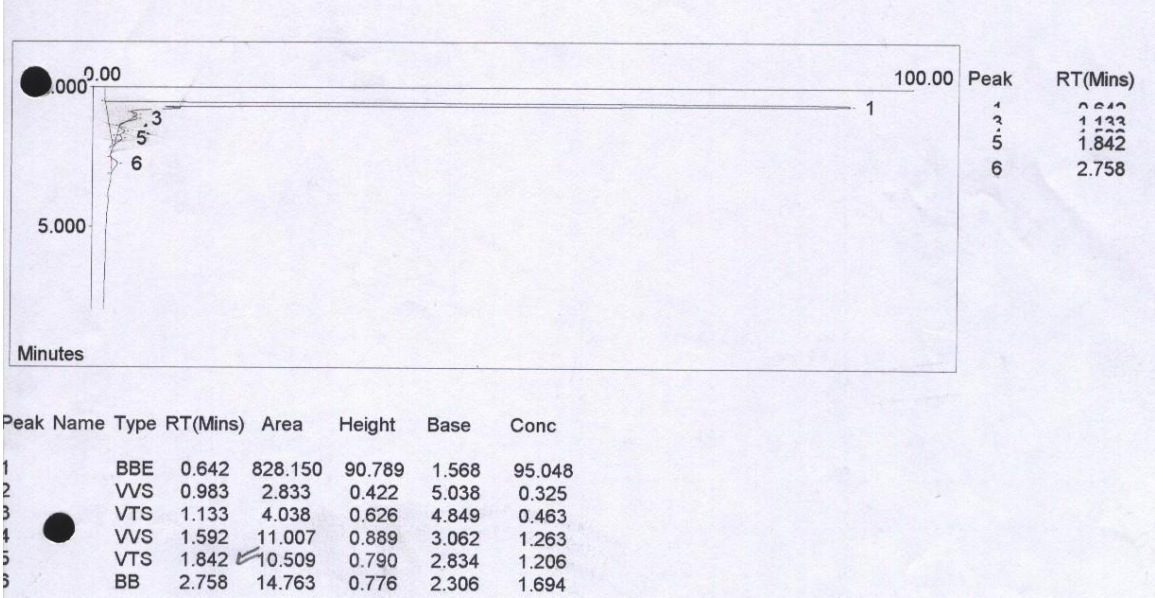
Sample identification: Karate MFKB-1, 0 day

Sample number: S_{26}

Analysis date: 28th April 2004

Amount found: ND

Appendix 10



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BBE	0.642	828.150	90.789	1.568	95.048
2		VVS	0.983	2.833	0.422	5.038	0.325
3		VTS	1.133	4.038	0.626	4.849	0.463
4		VVS	1.592	11.007	0.889	3.062	1.263
5		VTS	1.842	10.509	0.790	2.834	1.206
6		BB	2.758	14.763	0.776	2.306	1.694

Fresh leaves control sample

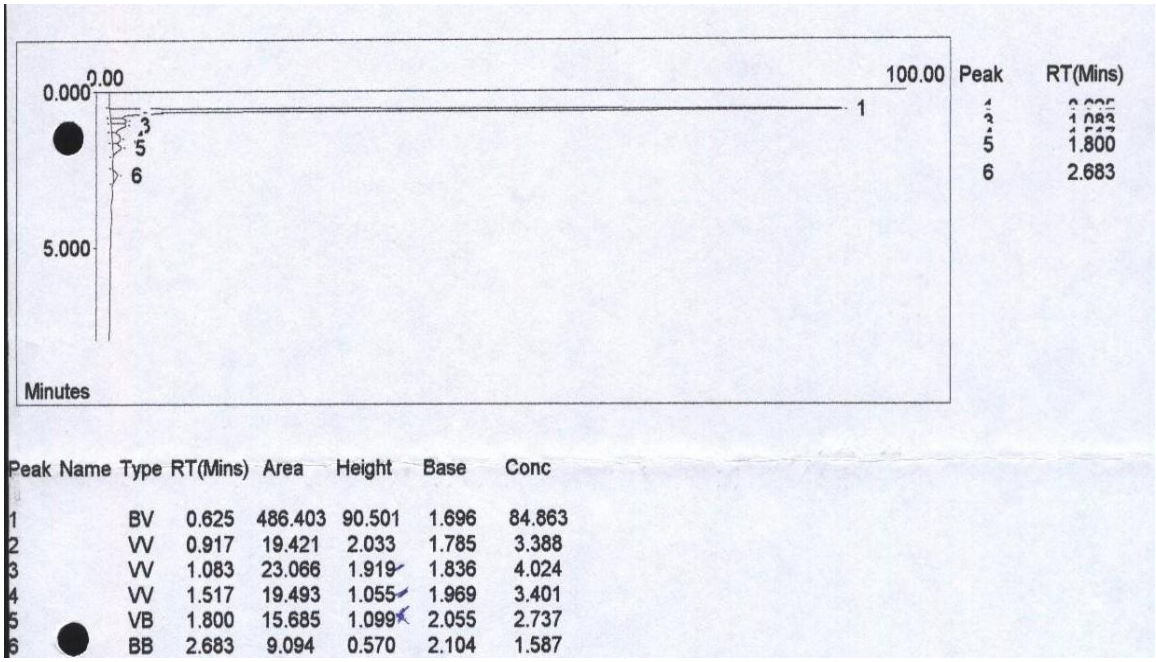
Sample identification: Pyagro control

Sample number: S_{37}

Analysis date: 30th April 2004

Amount found: 0.01ppm

Appendix 11



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BV	0.625	486.403	90.501	1.696	84.863
2		VV	0.917	19.421	2.033	1.785	3.388
3		VV	1.083	23.066	1.919	1.836	4.024
4		VV	1.517	19.493	1.055	1.969	3.401
5		VB	1.800	15.685	1.099	2.055	2.737
6		BB	2.683	9.094	0.570	2.104	1.587

Fresh leaves fortified sample

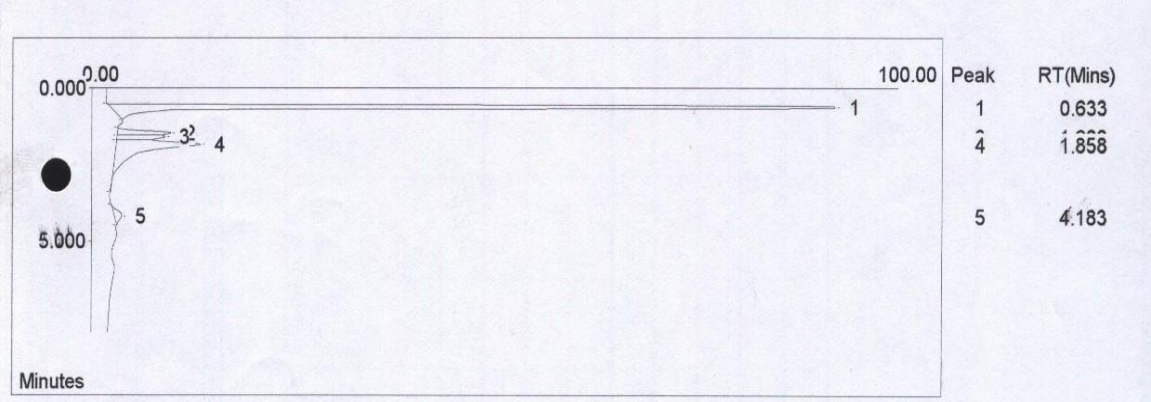
Sample identification: Pyagro PF-1

Sample number: S_{47}

Analysis date: 6th May 2004

Amount found: 0.05ppm (80% recovery)

Appendix 12



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BB	0.633	779.461	89.926	2.376	68.529
2		BV	1.475	59.017	6.761	3.205	5.189
3		VV	1.608	52.650	6.112	3.145	4.629
4		VT	1.858	224.598	10.616	3.031	19.746
5		BB	4.183	21.696	0.964	2.859	1.907

Fresh leaves treated sample

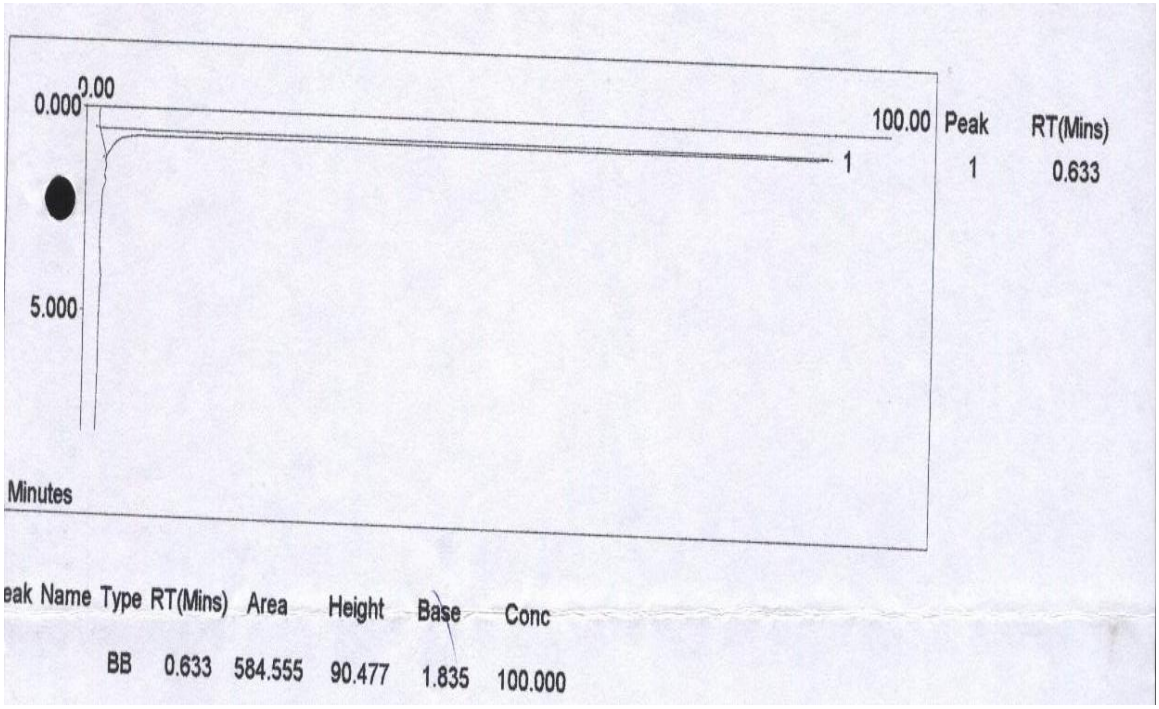
Sample identification: Pyagro 2.3R*, 0 day

Sample number: S_{39}

Analysis date: 3rd May 2004

Amount found: 1.07ppm (1.34ppm corrected)

Appendix 13



Black tea control sample

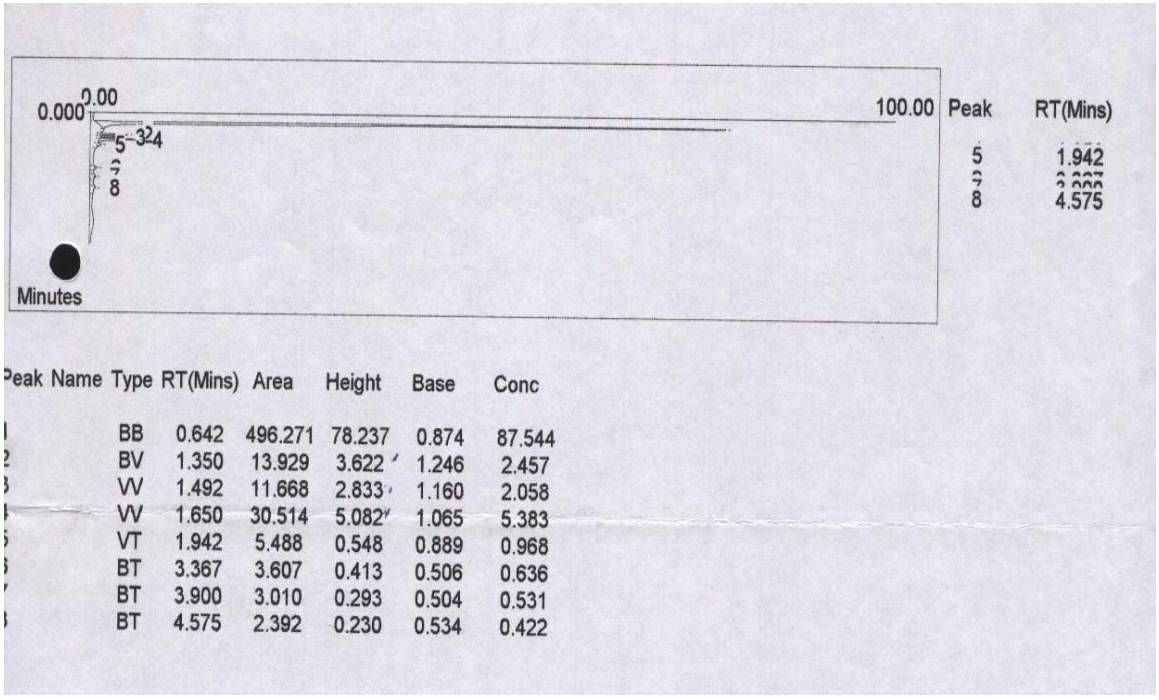
Sample identification: MFPyagro control

Sample number: S_{49}

Analysis date: 6th May 2004

Amount found: ND

Appendix 14



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
		BB	0.642	496.271	78.237	0.874	87.544
		BV	1.350	13.929	3.622	1.246	2.457
		VV	1.492	11.668	2.833	1.160	2.058
		VV	1.650	30.514	5.082	1.065	5.383
		VT	1.942	5.488	0.548	0.889	0.968
		BT	3.367	3.607	0.413	0.506	0.636
		BT	3.900	3.010	0.293	0.504	0.531
		BT	4.575	2.392	0.230	0.534	0.422

Black tea fortified sample

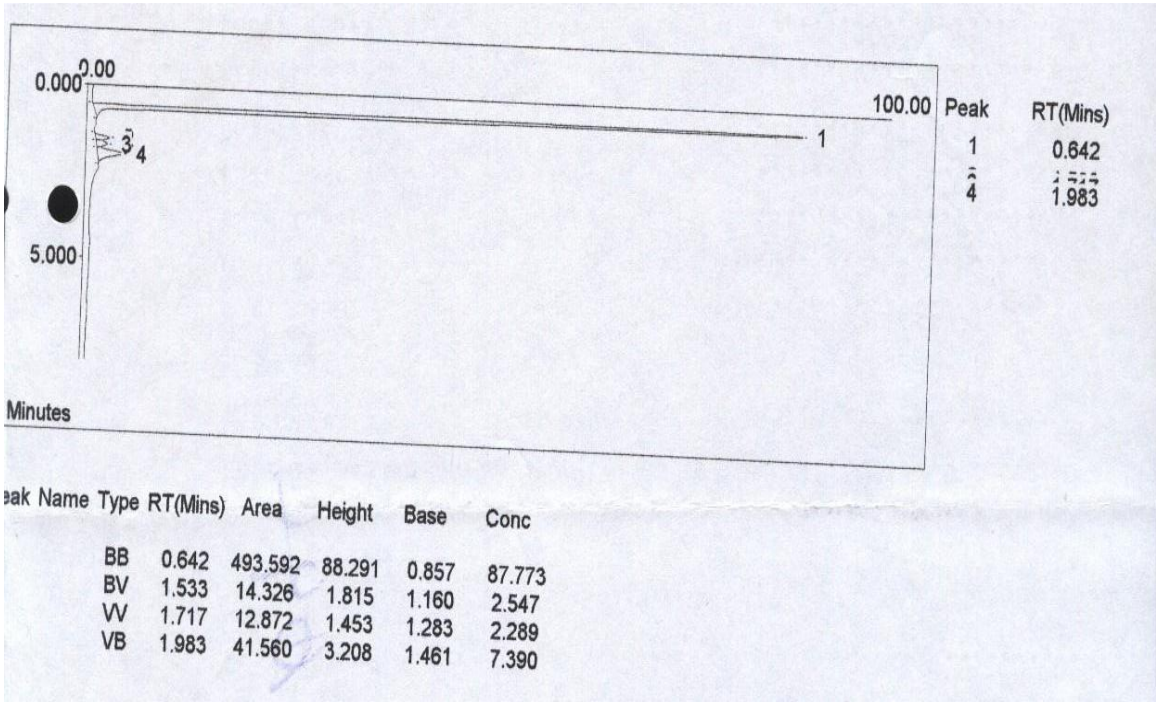
Sample identification: MFPyagro F-2

Sample number: S_{60}

Analysis date: 14^{nth} June 2004

Amount found: 4.98ppm (99.6% recovery)

Appendix 15



eak Name	Type	RT(Mins)	Area	Height	Base	Conc
	BB	0.642	493.592	88.291	0.857	87.773
	BV	1.533	14.326	1.815	1.160	2.547
	VV	1.717	12.872	1.453	1.283	2.289
	VB	1.983	41.560	3.208	1.461	7.390

Black tea treated sample

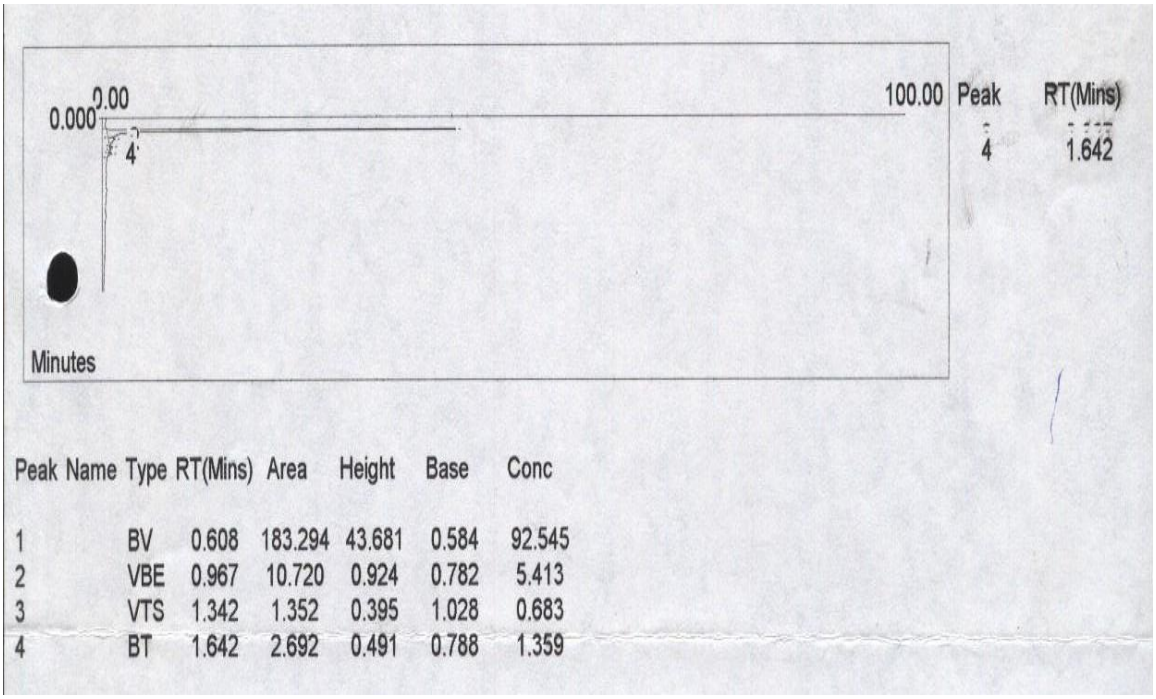
Sample identification: MFPyagro-1, 0 day

Sample number: S_{50}

Analysis date: 11^{nth} May 2004

Amount found: 0.199ppm (0.1998ppm corrected)

Appendix 16



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BV	0.608	183.294	43.681	0.584	92.545
2		VBE	0.967	10.720	0.924	0.782	5.413
3		VTS	1.342	1.352	0.395	1.028	0.683
4		BT	1.642	2.692	0.491	0.788	1.359

Brewed tea control sample

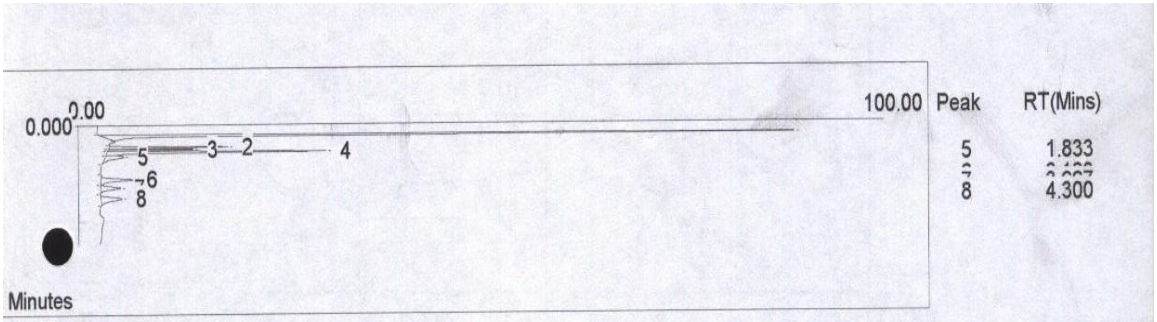
Sample identification: MFPyagroB-control

Sample number: S_{61}

Analysis date: 14^{nth} June 2004

Amount found: 0.0013ppm

Appendix 17



eak	Name	Type	RT(Mins)	Area	Height	Base	Conc
		BB	0.650	456.705	85.847	3.146	49.592
		BV	1.300	74.379	15.010	3.840	8.077
		VV	1.425	52.703	10.717	3.729	5.723
		VV	1.575	183.158	27.389	3.597	19.888
		VT	1.833	32.644	2.385	3.369	3.545
		BV	3.183	47.825	4.109	2.832	5.193
		VV	3.667	32.061	2.559	2.796	3.481
		VB	4.300	41.449	2.679	2.837	4.501

Brewed tea fortified sample

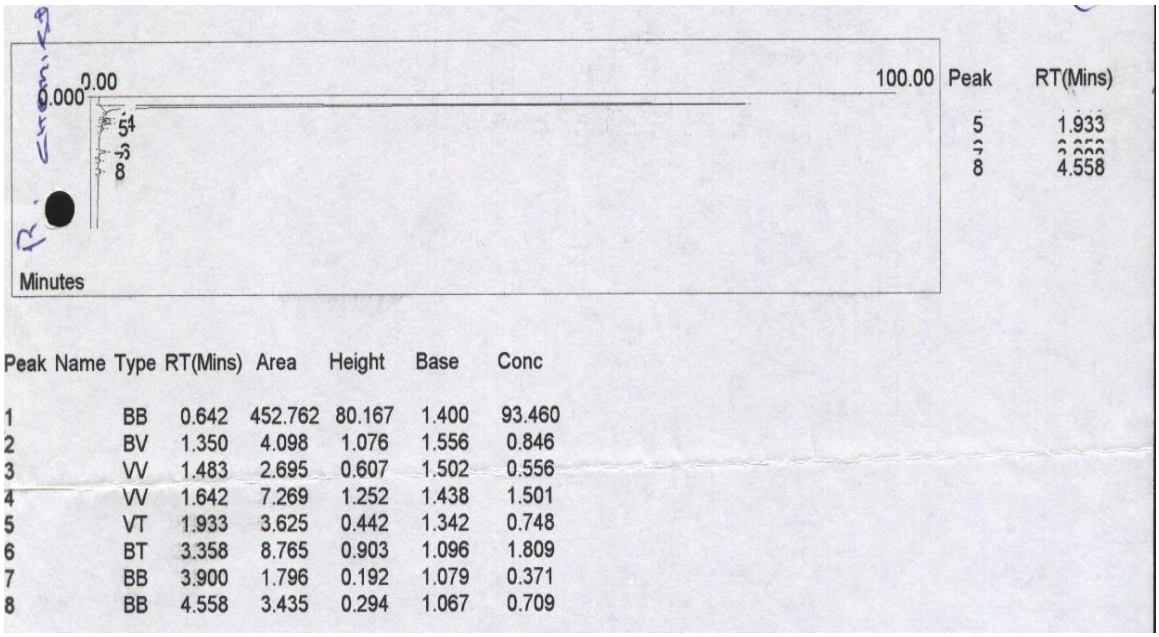
Sample identification: MFPyagroBF-2

Sample number: S_{72}

Analysis date: 16^{nth} June 2004

Amount found: 4.98ppm (99.6% recovery)

Appendix 18



Peak	Name	Type	RT(Mins)	Area	Height	Base	Conc
1		BB	0.642	452.762	80.167	1.400	93.460
2		BV	1.350	4.098	1.076	1.556	0.846
3		VV	1.483	2.695	0.607	1.502	0.556
4		VV	1.642	7.269	1.252	1.438	1.501
5		VT	1.933	3.625	0.442	1.342	0.748
6		BT	3.358	8.765	0.903	1.096	1.809
7		BB	3.900	1.796	0.192	1.079	0.371
8		BB	4.558	3.435	0.294	1.067	0.709

Brewed tea treated sample

Sample identification: MFPyagroB-1, 0 day

Sample number: S_{62}

Analysis date: 14^{nth} June 2004

Amount found: 0.032ppm (0.0328ppm corrected)

Appendix 19

EXAMPLE CALCULATION

Fresh Tea Samples and Black Tea

Initial sample weight =20 g

Aliquot taken =50g

Extraction solvent =100ml

Therefore g final weight $= \frac{20 \times 50}{100}$

$= 10g$

Brewed Tea Samples

Initial sample weight =20g

Aliquot taken = 150ml

Extraction solvent = 200ml

G final weight $= \frac{20 \times 150}{200}$

$=15g$

The final volume of the individual sample and the dilution factor will determine the mg injected.

Calculations for percentage recoveries of the fortified control samples were made with the following equation.

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

For a given set of samples of the value of percentage recoveries calculated for each fortified control were averaged, and for averages less than 100%, a correction factor was applied to the experimental samples for that set.

Examples

Karate Samples

a) Karate fresh tea leaf samples

Karate F-2 = 0.9557

% Recovery = 0.9557/1 X 100

=95.59

%age recovery not corrected for the control as the ppm found in control too high.

b) Black tea

Karate Control = 0.027ppm

Karate MFKF-1 = 0.0341ppm

Karate MFKF- 2 =0.8925ppm

$$\% \text{ recovery Karate MFKF-1} = \frac{0.0341 - 0.027}{0.01} \times 100$$

$$= 71\%$$

$$\% \text{ recovery Karate MFKF-2} = \frac{0.8925 - 0.027}{1} \times 100$$

$$=86.55\%$$

Average % recovery = (71 + 86.55) x ½

=78.8%

c) Brewed tea samples

Karate MFKBF-1= 0.0082

Karate MFKBF-1= 0.968

$$\% \text{ recovery Karate MFKBF-1} = \frac{0.0082}{0.01} \times 100$$

$$= 82\%$$

$$\% \text{ recovery Karate MFKF-2} = \frac{0.968}{1} \times 100$$

$$=96.8\%$$

Average of % recovery = (82+96.8) x ½

= 89.4%

Pyagro Samples

a) Pyagro fresh Samples

Pyagro Control = 0.01ppm

Pyagro F –1 = 0.05

Pyagro F- 2 = 0.002* (not used as it is too low)

Pyagro F –1 % recovery = $\frac{0.05 - 0.01 \times 100}{0.05}$

= 80%

b) Pyagro Black Tea

MF Pyagro Control =ND

MF Pyagro F-1 = 0.003 (not used as it is too low)

% recovery = 30%

MF Pyagro F- 2 = 4.98pm

% recovery = $\frac{4.98 \times 100}{5}$

= 99.6%

Not corrected for ppm in control as nothing was detected.

b) Pyagro Brewed Tea.

MF PyagroB Control = 0.0013ppm

MF PyagroB F1 = 0.049

MF PyagroB F2 = 4.98

% recovery MF PyagroB F1 = $\frac{0.049 - 0.0013 \times 100}{0.05}$

= 95.45%

% recovery MF PyagroB F2 = $\frac{4.98 - 0.0013 \times 100}{5}$

=99.6%

Average % recovery (95.4 + 99.60) ½

=97.55%

Appendix 20

KARATE PESTICIDE RESIDUE DATA SHEETS

Sample/standard identification	PHI	Fortif Level	µl inj	Fin vol	Aliquot (ml)	Dil factor	Peak height	ng found	mg inj	Ppm found	% recovery	Corr. ppm
Karate- control	0	-	2	7	50	2	10.39	1.287	1.43	*0.9	-	-
Karate 1.2	0	-	2	4	50	28	8.483	0.8064	0.1786	4.515		4.724
Karate 1.3	0	-	2	7	50	25	5.5285	0.5206	0.1143	4.555		4.766
Karate 1.4	0	-	2	4	50	3	1.465	0.134	1.67	0.0802		0.084
Karate 1.2	7	-	2	5	50	5	0.564	0.0505	0.4	0.1263		0.1322
Karate 1.3	7	-	2	5	50	2	6.96	0.6577	2	0.3289		0.3441
Karate 1.4	7	-	2	4	50	5	3.95	0.3692	1	0.3692		0.3863
Karate 1.1	14	-	2	7	50	2	3.659	0.3414	1.43	0.2387		0.2498
Karate1.2	14	-	3	0.5	50	25	4.0115	0.3751	1.6	0.2344		0.2453
Karate 1.3	14	-	2	7	50	2	2.482	0.2296	2.14	0.1073		0.1123
Karate KF-1	0	0.01	2	0.5	50	25	0.7305	0.0658	1.43	0.046	*460	
Karate KF-2	0	1	2	7	50	3	9.55	0.9102	0.9524	0.9557	95.57	
Karate MFKcon	0	-	3	7	50	3	0.435	0.0387	1.43	0.027		
Karate MFK-1	0	-	2	7	50	3	3.361	0.313	0.9524	0.3286		0.417
Karate MFK-2	0	-	2	7	50	3	3.94	0.3682	0.9524	0.3866		0.4906
Karate MFK-3	0	-	2	7	50	3	4.23	0.396	0.9524	0.4158		0.5277

Karate MFK-1	7	-	3	7	50	3	0.85	0.0768	1.43	0.053		0.067
Karate MFK-2	7	-	3	7	50	3	0.94	0.0851	1.43	0.0595		0.0755
Karate MFK-3	7	-	3	7	50	3	0.795	0.0717	1.43	0.0501		0.0636
Karate MFK-1	14	-	4	7	50	3	0.544	0.0487	1.905	0.0256		0.0325
Karate MFK-2	14	-	2	7	50	4	12.689					
Karate MFK-3	14	-	2	7	50	2	7.52					
Karate MFKF-1	0	0.01	2	5	50	2	0.757	0.0682	2	0.0341	71	
Karate MFKF-2	0	1	2	0.5	50	20	18.463	1.785	2	0.8925	86.55	
Karate MFKBC	0	-	4	5	50	1	ND					
Karate MFKB-1	0	-	4	5	150	1	ND					
Karate MFKB-2	0	-	4	5	150	1	ND					
Karate MFKB-3	0	-	4	5	150	1	ND					
Karate MFKB-1	7	-	2	5	150	1	ND					
Karate MFKB-2	7	-	4	5	150	1	ND					
Karate MFKB-3	7	-	4	5	150	1	ND					
Karate MFKB-1	14	-	4	5	150	1	ND					
Karate MFKB-2	14	-	4	5	150	1	ND					
Karate MFKB-3	14	-	4	5	150	1	ND					
KarateMFKBF1	0	0.01ppm	4	5	150	2	0.541	0.049	6.0	0.0082	81.67	
KarateMFKBF2	0	1ppm	4	5	150	9	10.391	1.287	1.33	0.968	96.8	

Appendix 21

PYAGRO PESTICIDE RESIDUE DATA SHEETS

Sample/standard identification	PHI	Fortif level	ml inj	Fin vol	Aliquot (ml)	Dil factor	Peak height	ng found	mg inj	Ppm found	% recovery	Corr. ppm
Pyagro control	0	-	4	5	50	1	1.837	0.0826	8	0.01	-	-
Pyagro 2.1R*	0	-	2	5	50	1	15.486	4.343	4	1.08	-	1.35
Pyagro 2.3R*	0	-	4	5	50	1	22.361	8.596	8	1.07	-	1.34
Pyagro 2.4R*	0	-	6	5	50	1	5.438	0.621	12	0.052	-	0.065
Pyagro 2.2	7	-	6	5	50	2	4.483	0.434	12	0.036	-	0.045
Pyagro 2.3	7	-	2	5	50	1	2.32	0.127	4	0.032	-	0.04
Pyagro 2.4	7	-	2	5	50	1	0.213	0.0015	4	0.0004	-	0.0005
Pyagro 2.1	14	-	2	5	50	1	ND	-	4	-	-	
Pyagro 2.2	14	-	2	5	50	1	ND	-	4	-	-	
Pyagro 2.3	14	-	2	5	50	1	0.286	0.0026	4	0.0007		0.0009
Pyagro PF1	0	0.05ppm	2	5	50	1	2.969	0.202	4	0.05	80	
Pyagro PF2	0	5ppm	2	5	50	1	0.522	0.008	4	0.002	*	
MFPyagro cont	0	-	2	5	50	1	ND	-	4	-		
MFPyagro –1	0	-	2	5	50	1	6.209	0.794	4	0.199		0.1998
MFPyagro –2	0	-	2	5	50	1	4.749	0.483	4	0.121		0.1249
MFPyagro –3	0	-	2	5	50	1	9.834	1.867	4	0.466		0.4679

MFPyagro –1	7	-	2	5	50	1	0.273	0.0024	4	0.0006		0.0006
MFPyagro –2	7	-	2	5	50	1	0.331	0.0034	4	0.0008		0.0008
MFPyagro –3	7	-	2	5	50	1	0.266	0.0023	4	0.0006		0.0006
MFPyagro –1	14	-	2	5	50	1	0.173	0.001	4	0.0003		0.0003
MFPyagro –2	14	-	2	5	50	1	ND	-	4	-		
MFPyagro –3	14	-	2	5	50	1	ND	-	4	-		
MFPyagro F-1	0	0.05ppm	2	5	50	1	0.188	0.0012	4	0.003	30	
MFPyagro F-2	0	5ppm	2	5	50	8	11.484	2.49	0.5	4.98	99.6	
MFpyagroB-cont	0	-	2	5	50	1	0.505	0.0075	6	0.0013		
MFPyagro B-1	0	-	2	5	150	1	2.909	0.194	6	0.032		0.0328
MFPyagro B-2	0	-	2	5	150	1	ND	-	6	-		
MFPyagro B-3	0	-	2	5	150	1	0.413	0.0052	6	0.001		0.001
MFPyagro B-1	7	-	2	5	150	1	1.189	0.036	6	0.006		0.0062
MFPyagro B-2	7	-	2	5	150	1	0.982	0.026	6	0.004		0.0041
MFPyagro B-3	7	-	2	5	150	1	1.673	0.069	6	0.012		0.0123
MFPyagro B-1	14	-	2	5	150	2	0.691	0.0134	3	0.0045		0.0046
MFPyagro B-2	14	-	2	5	150	1	1.188	0.037	6	0.0062		0.0064
MFPyagro B-3	14	-	2	5	150	1	1.612	0.065	6	0.0108		0.0111
MFPyagro BF-1	0	0.05ppm	2	5	150	10	1.051	0.029	0.6	0.049	95.4	
MFPyagro BF-2	0	5ppm	2	5	150	1	43.755	29.94	6	4.98	99.6	