DIETERY RISK ASSESSMENT FOR EXPOSURE TO PESTICIDE RESIDUES IN EXOTIC VEGETABLES SOLD IN NAKURU TOWN, KENYA

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Degree of Master of Science in Environmental and Occupational Health of Egerton University

EGERTON UNIVERSITY

OCTOBER, 2019

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not been presented for examination in any institution for the award of any other degree.

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Recommendation

This thesis has been submitted for examination with our approval as university supervisors.

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DEDICATION

This work is dedicated to my father Mr. Simeon Kenyanya, my mother Esther Nyaboke, my siblings Job, Cynthia, Faith and Marion and my husband Erick for their constant support and prayers.

ACKNOWLEDGEMENT

I would like to thank the Almighty God for the gift of life and good health throughout the research period. I would also thank Egerton University for the opportunity to study and utilise their facilities. I would like to appreciate my supervisors Prof. Wilkister N. Moturi and Dr. Jane Nyaanga who were dedicated in guiding me and for their contribution to this work. I am also grateful to Egerton Alumni Association and Ecological Organic Agriculture Initiative (Egerton university) for their assistance in the acquisition of laboratory chemicals. I would also like to appreciate Mr David Samoei of University of Eldoret for his assistance in guiding me on how to operate HPLC machine. Finally my parents, husband, siblings and friends for their support throughout the period of this work. God bless you all.

ABSTRACT

Vegetables constitute an important part of the human diet as a source of nutrients such as vitamins. The emerging lifestyle changes and need to live a healthy life have led to increased consumption of vegetables. There are, however, growing concerns over the indiscriminate use of pesticides and lack of clear safety standards for maximum residue levels for vegetables sold in the local market. This study was done to assess human exposure to pesticide residues through consumption of exotic vegetables sourced within Nakuru County and sold in selected markets in Nakuru town. The study used cross sectional study design. It involved social survey using structured questionnaires on consumers, farmers and stakeholders and laboratory analysis of vegetable samples for pesticide residues. Structured questionnaires were used to get data on sources and consumption rates of most consumed vegetables and most commonly used pesticides. Sample extraction and residue analysis was done using AOAC official method 2007.01. Pesticides residue analysis was done using Reverse-phase High Performance Liquid chromatography. Peak areas of the curves were calculated using Motic Images plus 2.0 and recovery rates of pesticides were recorded. The hazard quotient and hazard index was also calculated. Frequencies were used to analyse data on most commonly used pesticides which were cypermethrin and lambda-cyhalothrin. Mean was used to analyse date on most consumed exotic vegetables which were Kales (*Brassica oleracea var. acephala*), Tomatoes (*Solanum lycopersicum*) and Spinach (*Beta vulgaris subsp. cicla*). Hazard quotients and indices were calculated to determine risk of exposure to the pesticide residues. Recovery rates of pesticides in the laboratory analysis ranged from 87.78% to 97.93% for cypermethrin and 90.65% to 95.72% for lambda-cyhalothrin. Mean residue concentration levels in samples ranged from 2.495mg/kg to 0.238mg/kg for cypermethrin and 0.352mg/kg to 0.119mg/kg for lambda-cyhalothrin. Most of the hazard quotients and hazard index value computed of the two pesticides were within the recommended value ≤ 1. It is recommended that farmers should be encouraged to adhere to Pre-harvest interval after spraying pesticides to reduce residue levels. KEPHIS, KEBS and Ministry of agriculture, fisheries and livestock should also set and monitor regularly maximum residue levels of pesticides in locally consumed commodities to protect the consumers.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADI: Acceptable Dietary Intake

ATSDR: Agency for Toxic Substances and Disease Registry

BW: Body Weight

CAC: Codex Alimentarius Commission

EDI: Estimated Daily Intake

EFSA: European Food Safety Authority

FAO: Food and Agriculture Organization

g: Grams

HI: Hazard Index

HPLC: High Performance Liquid Chromatography

HQ: Hazard Quotient

IARC: International Agency for Research on Cancer

IFCS: Intergovernmental Forum on Chemical Safety

JMPR: Joint FAO/WHO Meeting on Pesticide Residue

KEPHIS: Kenya Plant Health Inspectorate Service

Kgs: Kilograms

KEBS: Kenya Bureau of Standards

KNBS: Kenya National Bureau of Statistics

Mg: Milligram

MoA: Ministry of Agriculture

MRLs: Maximum Residue Levels

PCPB: Pest Control Products Board

UNCED: United Nation Convention on Environment and Development

UNDP: United Nations Development Programme

UNEP: United Nations Environment Programme

USDA: United States Department of Agriculture

USEPA: United States Environment Protection Agency

USFDA: United States Food and Drug Administration.

WHO: World Health Organization

CHAPTER ONE
INTRODUCTION

1.1. Background information

Vegetables contain nutrients such as Vitamin A, C and E, phytochemicals such as zeaxanthin and minerals such as folic acid, phosphorous and zinc among others that are important to health (Kader *et al*., 2012, Heneman *et al*., 2008). Human health benefits of vegetables have become popular and this has led to an increase in their consumption over the years. Production of vegetables globally has been on the rise as different organizations such as the World Health organization encourage people to increase their daily vegetable intake

Vegetable production in Kenya has been on the rise over the years as there is also a ready market for the products. Exotic and indigenous vegetable farming have gained popularity both in rural and urban areas. This is evident as seen among urban dwellers that practice sack-farming since land is limited in such areas. Production of vegetables in Nakuru town and its environs has increased as their market is on expansion. Being the fastest growing town in Kenya, there is an influx of human population which has attracted more business opportunities. This has seen the establishment of businesses such as supermarkets that have increased in number hence contributing to expansion of the existing market. These have raised demand for vegetables as they are also relatively affordable by consumers.

Pests and plant diseases are a challenge to vegetable growers and a threat to global food security. Pests are organisms that feed on plants as a source of food (Ata *et al*., 2013) and in the process destroy the crops and reduce their yield. Pests can also be carriers of plant pathogens which also exist in soil. Farmers use pesticides to control the pests and plant diseases. Pesticides have been in use since early years by Sumerians where they used compounds of sulphur to control mites and insects 4500 years ago. They also used mercury, lead, zinc and arsenical compounds to grow vegetables and fruits (Unsworth, 2010). Before the invention of chemical industries, most pesticides were derivatives of plants and animals (Fishel, 2013).

Chemical pesticides are widely used globally as they are perceived to be more effective and relatively cheap. Kenya being a country that widely relies on agriculture, there is widespread use of chemical pesticides to control plant diseases and pests. This is in effort to improve yield and produce blemish-free products. Emergence of new diseases and pests has led to the need of better pesticides to counter this problem. Farmers are encouraged to embrace new methods of controlling plant diseases and pests such as integrated pest management to reduce reliance on chemical pesticides. Despite this effort, use of chemical pesticides is still wide spread in the country.

Bio-magnification also known as biological magnification is the increase in concentration of chemicals in organisms as one moves from lower to higher trophic level. Pesticides that can be bio-magnified are mostly lipophilic such as DDT, carbaryl and proporxur (Bonita, 2015). Biomagnification of these pesticides occurs because they either cannot be excreted from the body easily or their rate of degradation is slower. For example, when DDT is sprayed on or carried by surface runoff to aquatic ecosystem, it is fed on by it is taken in by phytoplanktons and zooplanktons which are subsequently fed on by small fish then big fish. Therefore, any organisms including human beings who feed on big fish take higher concentrations of DDT. The high concentration taken can affect human health.

Bioaccumulation also known as biological accumulation is the build up in concentration of chemicals in organs of an organism over a period of time. Chemical pesticides can accumulate in the human body organs especially the lipophilic ones. Human beings can be exposed to pesticides from different sources such as vegetables and meat from animals that have feed on feeds containing pesticides. The chemicals from these different source take longer in the human body resulting to their accumulation. Accumulation of these chemicals can cause harm to human health over a period of time.

Several cases on effects of pesticides have been reported, for example; in 1958, all members of a local chief's family in Nigeria who were cocoa farmers got hospitalized after feeding on a leaf vegetable that was an undergrowth after spraying the cocoa with lindane. In another incident in 2004, residues of carbofuran were found on noodles manufactured in Nigeria and they were reported to have caused 23 cases of vomiting and one death (Monosson, 2011). Apart from these specific cases, the WHO estimates that there are 3 million cases of pesticide poisoning and 220,000 deaths each year (WHO, 2008).

Some pesticides are banned both internationally and locally. Locally, for example DDT and dimethoate have been banned for us in vegetables. However, a study done in lake Naivasha basin showed that there is still use of banned pesticides such as endosulfan trading in different names (Njogu, 2014). Another study done among tomato farmers in Kathiani showed that there is use of dimethoate (Mutuku *et al*., 2014a). Dimethoate was banned in 2012 by the government of Kenya (MoA, 2012). Continuous use of banned pesticides in the country can be attributed to weak regulatory framework on pesticides and inadequate capacity to carry out surveillance on pesticides being imported and used in the country.

1.2. Statement of the problem

Farmers use pesticides to control pests and disease to improve yields and quality of their products. New plant diseases have been experienced in different parts of the country as a result of climate change. In addition, there is increased demand of vegetables as people seek to maintain healthy lifestyles. At the same time, consumers also demand for products that are of good quality and blemish free. To respond to the expanding market demand, vegetable growers have drastically changed their farming practices to fulfil the needs of their customers with not just the best but with consistent quality and quantity. This has resulted in indiscriminate use of pesticides to manage pests and diseases. Most pesticides have a pre-harvest period after application of pesticides but most farmers do not observe this period hence the likelihood of harvesting vegetables that have pesticide residues. Consumers are therefore at a risk of being exposed to high pesticides levels in their vegetables. Various studies have indicated the link between pesticide residues to negative health effects in human beings such as cancer (Berrada *et al.*, 2010). Consumption of such vegetables with pesticide residues can expose people and increase their risk to such health effects. This study was therefore done to assess human exposure to pesticides through consumption of exotic vegetables. The research findings were aimed at creating awareness to the general public and the relevant authorities on the need to monitor pesticide residues in locally consumed products for the protection of public health.

1.3. Objectives

1.3.1. Broad Objective

To assess human exposure to pesticide residues in exotic vegetables sold in selected markets in Nakuru town.

1.3.2. Specific Objectives

1. To find out three most consumed exotic vegetables sourced within Nakuru County sold in Nakuru town and their consumption rates.

2. To document market chain and handling practices of three most consumed exotic vegetables sourced within Nakuru County sold in Nakuru town.

3. To determine the concentration of most commonly used pesticides in the vegetables sold in Nakuru town from their sources (areas of production).

4. To estimate the risk of exposure to most commonly used pesticides through consumption of the selected vegetables sold in selected markets of Nakuru Town.

1.4. Research Questions

1. What is the consumption rate of three most consumed exotic vegetables sourced within Nakuru County

2. What is the market chain of three most consumed exotic vegetables sourced within Nakuru County sold in Nakuru town?

3. How are the vegetables handled by consumers before consumption?

4. Which are the most commonly used pesticides and what are their chemical components and properties?

5. What is the concentration of the most commonly used pesticides in the vegetables?

6. What is the estimated risk of exposure to pesticide residues through consumption of the vegetables?

1.5. Justification

In Kenya, pesticide residues levels in vegetables are commonly monitored for export products. However, monitoring of pesticide residue levels in vegetables consumed locally is insufficiently done. This could be attributed to lack of adequate resources, weak regulatory framework and ignorance from consumers. There is inadequate documented data on pesticide residue levels for locally consumed horticultural products. Nakuru being an agricultural town in Kenya, it is conducive for the production of vegetables both for local consumption and exports. The area is also frequented by tourists, hence there is need to know the quality of vegetables sold locally to protect them and citizens. Analyzing for pesticide residue levels in the selected vegetables would give a good indication on the exposure of consumers to pesticides through consumption.

Even though pesticide residues in foods is not directly mentioned in sustainable development goals, it is an element of safe food in the first target of goal two which states that "By 2030, end hunger and ensure access by all people, in particular the poor and people in vulnerable situations, including infants, to safe, nutritious and sufficient food all year round"(UNDP, 2015). Data from this study will help in providing baseline information for future development of monitoring strategy of pesticide residue levels for locally consumed vegetables for the protection of human health. It can also be used by entities concerned with food safety such as the Department of public health and Kenya bureau of standards.

1.6. Scope

The study was done in Nakuru town which has two constituencies, that is, Nakuru Town West and Nakuru Town East constituencies. Markets from the two constituencies were purposely selected because of their sizes and the large population they serve. Soko-Mjinga market is in Nakuru Town West constituency, Ponda-Mali market is a trans-boundary market shared by the two constituencies while Main Municipal Market market is the main market in Nakuru town located in Nakuru Town East constituency. The markets also serve as distribution points in Nakuru town. The study involved Key informants, farmers, vendors from whom vegetable samples were bought from and consumers. Laboratory analysis was done at University of Eldoret in Biotechnology laboratory. The study only covered exotic vegetables that are perceived to be sprayed more with pesticides compared to indigenous vegetables. The analysis was done on raw vegetable samples bought from the selected markets, they were not washed. Even though consumers were asked about how the handle vegetables before consumption, the handling practices were not tested in the laboratory to determine the effects on pesticide residues. The practices were however discussed and how they affect pesticide residue from other studies.

1.7. Assumptions

1. All respondents consume vegetables from one or more of the selected markets.
2. All samples of the vegetables are not from same source.
3. Respondents of questionnaires will be able to recall and answer the questions accurately.
4. All the vegetable samples included in the analysis were conventionally grown.
5. All farmers use the pesticides that had been mentioned by farmers included in the study.

1.8. Limitation

There was language barrier in some among some respondents especially in Subukia sub-county. This was overcome by the help of the agricultural extension officer who understood the local language

1.9. Definition and operationalization of terms

Clean-up: refers to series of steps in the analytical procedure in which the bulk of the potentially interfering co-extractives are removed by physical or chemical methods.

Composite sample: a homogenous mixture of a sample.

Consumption Rate: the amount in kilograms of selected vegetables consumed per day.

Extraction: this is the separation of pesticide residues from a mixture using solvent

Exotic vegetables: These are vegetables that are not native to Kenya

Market chain: This includes production sources and supply points of vegetables to consumers.

Maximum residual levels: the maximum concentration of a residue that is legally permitted and acceptable on/in food or agricultural commodity.

Pesticide: a chemical substance or mixtures of chemical substances that are used to control or kill pest and control disease that affect vegetables.

Pesticide residue: a remaining pesticide after spraying or its metabolites found on vegetables after a certain period of time.

Human exposure: coming into contact with pesticide residue through consumption of vegetables.

Handling practices: how consumers treat vegetables before they consume. For this study, the practices will be limited to washing and cooking.

CHAPTER TWO

LITERATURE REVIEW

2.1. Introduction

A pesticide is any substance or mixture of substances intended for preventing, destroying, or controlling any pest including vectors of human or animal diseases, unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, or marketing of food, agricultural commodities, wood and wood products, or animal feedstuff, or which may be administered to animals for the control of arachnids or other pesticide in or on their bodies. UNEP and FAO (1990), defined pesticide as chemicals that are designed to combat attacks of pests and vectors on crops, domestic animals and human beings. Since pesticides are used to control noxious and unwanted living species (Baxter *et al*., 2010), they find their application in agriculture, in industry to protect machineries and products from biological degradation, in public health for controlling vector borne disease and other activities such as gardening (Collotta *et al*., 2013).

2.2. Consumption of vegetables

Consumption of vegetables is wide spread among most people in the world. According to WHO (2003), fruits and vegetables consist an average of 30% (based on mass) of food consumption and are the most frequently consumed food group. Consumption of vegetables differs across groups of people depending on locality, availability and cultural practices. The World Health Organisation recommends a minimum intake of 400g of vegetables per person per day or 146kg/person/year. However, a survey done in 21 countries in 2003 showed that only three countries that is, Israel, Italy and Spain had reach the minimum recommended level (IARC, 2003). Vegetable consumption was projected to improve over the years as more people become aware of the importance of consuming vegetables. Consumption of vegetables in Kenya in 2005 was estimated to be 88.3kg/person/year which is way below the minimum recommended intake (Ruel *et al.*, 2005) There is inadequate information on consumption rates of exotic vegetables in Kenya. According to Hamilton *et al*., (2004), food consumption data can be obtained by conducting food supply surveys, household inventories, household food use and individual food intake. There are various methods of dietary assessment methods which include; food frequency, dietary records, diet history and dietary recall (EFSA, 2009). Food consumption data can be collected using surveys based on food recall method (Qaim, 2014, Agudo, 2006)

Vegetables provide nutrients that are vital for human health. They contain Vitamin A and C, potassium, dietary fibre, antioxidants and folate. Vegetables rich in potassium help to maintain blood pressure reduce the risk of kidney stones and decreases bone loss. Dietary fibre is important as it helps in reducing blood cholesterol levels, lower the risk of heart disease, obesity and type II diabetes. Fibre is also important for proper functioning of bowel, reduces constipation and diverticulosis. Vitamin A helps in keeping eyes and skin healthy and protects them against infections. On the other hand, Vitamin C helps in healing cuts and wounds and keeps gums and teeth healthy. It also helps in iron absorption in the body. Folate helps in the formation of red bloods cells in the body. It is also important to expectant women for reducing risk of birth defects such as neural tube defects, spina bifida and anencephaly as the foetus develops (USDA, 2015). Vegetables contain a group of antioxidants that are important in fighting cellular damage and helps in preventing cancer, heart disease, Parkinson disease, atherosclerosis and Alzheimer,s disease (Hyland-Tassava, 2013).

2.3. Classification of pesticides

Globally harmonized system of classification and labeling of chemicals (GHS) provides guidelines on classification of chemicals and their labeling. The system classifies chemical in terms of physical hazards, effect on human health and effects on environment (United Nations, 2011).

According to Louis (1994) and Buchel (1983), pesticides can be classified according to chemical composition as organochlorines, organophosphates, carbamates and pyrethroids or pyrethrins. Organochlorines are organic compounds with five chlorines atoms within its structure such as lindane, endosulfan, aldrin and chlordane. Organophosphates on the other hand contain a phosphate group within its structure. Unlike organochlorines, Organophosphates degrade easily through chemical and biological actions in the environment hence they are not persistent. According to WHO (2009) pesticides can be classified based on toxicity as extremely hazardous (IA), highly hazardous (IB), moderately hazardous (II), slightly hazardous (III), unlikely to present acute hazard (U). Pesticides can also be classified as insecticides, biopesticides, fungicides and others (Kodandaram *et al*., 2013).

Pesticides can be classified based on target pest species. The word usually has a suffix – cide which means to kill. Such pesticide include: insecticides, herbicides, fungicides, miticides, rodenticides, piscicides and nematicides among others. These pesticides are used to control and kill insects, weeds, fungi, mites, rodents, fish and nematodes respectively. Pesticides can also be classified or grouped on their mode of action. In this classification system, pesticides are classified as contact pesticides, systemic pesticides, foliar pesticides, soil-applied pesticides, fumigants, preplant herbicides, premergent herbicides, postmergent herbicides, translocated herbicides, eradicant fungicides, protectant fungicides, selective pesticides, non-selective pesticides, suffocating insecticides, residual pesticides and non-residual pesticides. Other classifications include classification by mode of action, chemical structure, pesticide formulations and activity spectrum that is either broad spectrum or narrow spectrum. (Ministry of Agriculture, 2017). Pesticides can also be classified or grouped on their mode of action. In this classification system, pesticides are classified as contact pesticides, systemic pesticides, foliar pesticides, soil-applied pesticides, fumigants, preplant herbicides, premergent herbicides, postmergent herbicides, translocated herbicides, eradicant fungicides, protectant fungicides, selective pesticides, non-selective pesticides, suffocating insecticides, residual pesticides and non-residual pesticides. Other classifications include classification by mode of action, chemical structure, pesticide formulations and activity spectrum that is either broad spectrum or narrow spectrum (Ministry of Agriculture, 2017).

2.4. Commonly used pesticides in vegetables

Over the years farmers have been using pesticide to improve their yield and protect their crops from destruction. Pesticides can be named using trade or brand names that are suggested by companies and submitted to International Standards Organization. They can also be named using common names and chemical or systematic names. Chemical pesticides usually have long scientific chemical names also known as systematic names depending on chemicals used as ingredient. A pesticide is usually named after an active ingredient used to make the pesticide. Apart from active ingredient, chemical pesticides may be accompanied with other chemical ingredients known as inert ingredients or adjuvants. These ingredients may help in prolonging shelf-life, make the pesticide smell better or carry the active ingredient within spray, dust or gel for easy application (Wood, 2015). Systematic names are derived using set either by The International Union of Pure and Applied Chemistry (IUPAC) or Chemical Abstracts Service. Systematic names are long and difficult to remember. Therefore, chemical pesticides are given common names. Common names are easy to remember, promote user understanding of chemical-based products, provide a ready reference for people who have inadequate technical and scientific background and fosters informed choice while using and purchasing these products (USEPA, 2015). Common names are given by a committee within International organization for Standardization (ISO).

According to Fishel (2014), physical and chemical characteristics of a pesticide determine its interactions within the environment. Physical characteristics include colour, odour, solubility in water, vapour pressure and molecular weight and form. In a study done by Keikotlhaile, (2011), on presence of pesticide residues in vegetables in Belgium, established that pesticides can undergo volatilization, photolysis, chemical and microbial degradation.Most commonly used pesticides in vegetables include chlopyriphos, dimethoate, diazinon and chlorothaloni (KEPHIS, 2013a). According to Inonda *et al*., (2015), synthetic pyrethroids such as cyhalothrin and cypermethrin are commonly used by farmers in vegetables. The study also showed that organochlorines and carbamates are rarely used on vegetables. There is inadequate information of the most used pesticides in the study area.

2.5. Synthetic Pyrethroids

Synthetic Pyrethroids are derivatives of pyrethrins that are extracted from natural pyrethrum and consist a group of approximately one thousand insecticides. Despite them being derivatives of pyrethrins, their production involves chemical modification that are more extensive. This chemical process makes synthetic pyrethroids more toxic and less degradable in the environment than the original pyrethrins. They are categorised in two groups namely; Type I and Type II based on their physical and toxicological characteristic. Type I have a cyano group and can educe tumors while type II have a cyano group and can cause chloreoathetosis and salivation. The pyrethroids are composed of two, four or eight isomers. Toxicity of same compound of pyrethroid may vary due to isomers ratio in individual pyrethroids (ATSDR, 2003).

Table 2.1: Types of Pyrethroids

Type I Pyrethroids	Type II Pyrethroids
Allethrin	Cyfluthrin
Bifenthrin	Cyhalothrin
Permethrin	Cypermethrin
Phenothrin	Deltamethrin
Resmethrin	Fenvalerate
Tefluthrin	Fenpropathrin
Teramethrin	Flucythrinate
	Flumethrin
	Fluvalinate
	Tralomethrin

Source: ATSDR, 2003.

2.5.1. Lambda-cyhalothrin

Lambda-cyhalothrin (α-cyano-3-phenoxybenzyl 3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropanecarboxylate) belongs to type II group of synthetic pyrethroids. It is a broad spectrum insecticide which has found extensive uses in agriculture, public and animal health where it is used in controlling cockroaches, flies, lice, ticks and mosquitoes (Davies *et al.*, 2000, Kroeger *et al.*, 2003). It has a molecular weight of 449.9g/mol, melting point of 49.2 °C and decomposition temperature of 275 °C.

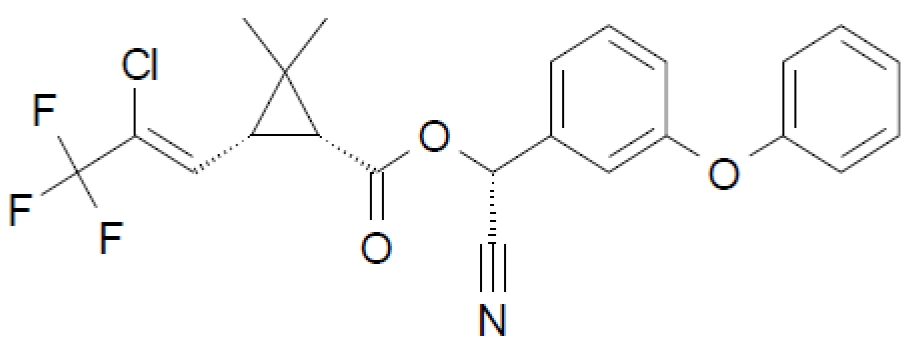

Figure 2.1: Molecular Structure of Lambda-Cyhalothrin

Source: ATSDR,2003.

3.3.1. Cypermethrin

Cypermethrin is a non-systematic synthetic pyrethroid insecticide with a wide range of agricultural applications including the control of ectoparasites. It degrades in soil and sediments quickly with photolysis and hydrolysis playing major role in the degradation. It has low solubility in water but highly soluble in organic solvents like acetone and hexane. It has a molecular weight of 416.30, melting point of 80.5 °C and boiling point of 200 °C (Crane *et al.*, 2007).

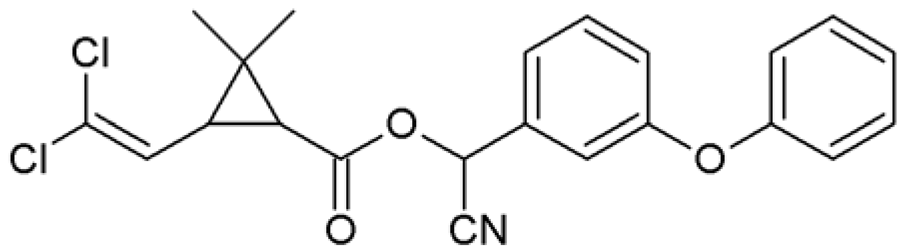

Figure 2.2: Molecular Structure of Cypermethrin

Source: ATSDR, 2003.

2.6. Pesticide Residues and Maximum Residual Limits for Pesticides

In the environment, pesticides can breakdown through physical and chemical means to form other products. This affects the original concentration of pesticides and results to presence of metabolites. Pesticide metabolites are also used in determination of pesticide residue since some are toxic to people and animals. In a study done in Ghana, pesticide residue of selected pesticides on selected fruits and vegetable were found to exceed MRLs set by WHO (Bempah *et al*., 2011). It was also established that banned pesticides such as DDT are used in Ghana. Pesticide residues in one-third of samples of major stable foods in Ethiopia were found to be above MRLs (Mekonen *et al*., 2014).

In another study conducted in Lebanon indicated that 55% of the samples collected and analysed were found to have one pesticide residue while 45% of the samples were found to have 2-4 residues. The pesticides that were detected included chlopyrifos, procymidone, primiphos methyl, dimethoate and dieldrin (Nasreddine *et al.,* 2002). Residues of organochlorine pesticides such as hexachlorobenzene, DDT, Chlordane compounds, hexachlorocyclohexane and heptachlor were detected in sampled foods in a study done in china. Even though they were detected, they did not exceed the MRLs (Zhou *et al.,* 2012). A study done in Pakistan by Aamir *et al.,* (2018) on dietary exposure to DDT and Hexachlorocyclohexane (HCH) found out that there were residues of the pesticides in both food of animal origin and vegetable origin. The study also associated the risk of cancer with consumption of foodstuffs contaminated with DDT and HCH.

Monitoring of Pesticide residues in Kenya on foods and agricultural products is done by KEPHIS for exports (European Commission, 2014). According to KEPHIS (2013), pesticide residue levels were analysed for export produce, locally consumed and industrial produce. It was found that pesticide residues levels in some locally consumed products exceeded MRLs standards. A study done by Kithure *et al*., (2017) on fate of lambda-cyhalothrin in kales, tomatoes and cabbage found out that the residues of the pesticide in the vegetables were below the MRLs. In another study on deltamethrin residues in kales, cabbage and tomatoes, found out that the residues of the pesticide were below the MRLs (Kithure *et al*., 2014). However, in a study done by Inonda *et al*., (2015) found out that various pesticide residues in kales and French beans exceeded the MRLs.

Maximum Residual Levels refers to maximum concentration of a residue that is legally permitted or recognized as acceptable in or on a food or agricultural commodity or animal feedstuff (FAO, 2005a). According to EFSA (2010), Maximum residue levels are the highest levels of residues expected to be in/on food when pesticide is used according to authorized good agricultural practices. Pesticide residue is defined as any specified substances such as derivatives of a pesticide and metabolites in or on food, agricultural commodities or animal feed resulting from use of pesticide (FAO, 2005). In setting MRLs, marker residues are used. According to JMPR, a marker residue is a residue whose concentration decreases in known relationship to the level of total residues in or on foods. In doing this, a pesticide labelled with radioactive isotope is used and results of the determinations of total residue (total radioactivity) are compared with the concentrations of marker residue. These studies are carried by JMPR in selecting pesticides used on food and residue(s) that are used in dietary risk assessment and on which MRLs are set by CAC (FAO/WHO, 2006).

2.7. Effect of handling practices and environmental factors on concentration of pesticide residues

Handling practices of food stuff before consumption affects the amount of pesticide residues that one can be exposed. According to USFDA, (2016) vegetables have to be thoroughly washed using clean running water. This is done to remove dirt and bacteria on the surface of vegetables that might cause diseases. Handling practices such as washing with clean running water have been found to reduce pesticide residue in vegetables. A study done by Selim *et al.,* (2011) on the effect of processing vegetables in households on pesticide residues and found out that washing sweet pepper with tap water reduced the concentration of cypermethrin, dicofol, endosulfan, metalaxyl, pirimiphos-methyl, dimethoate, and methomyl by 65%, 67%, 49%, 30%, 10%, 15% and 59% respectively. The same study also found out that adding acetic acid to water used to wash vegetables reduced the residues further for methomyl (99.7%), dimethoate (34%), pirimiphos-methyl (89%), metalaxyl (61%), endosulfan (90%), dicofol (100%), and cypermethrin (100%). In another study done by Bonnechère *et al.*,(2012) found out that residues of boscalid, chlorpyrifos, tebuconazole, dimethoate, difenoconazole and linuron in carrots reduced by up to 90% after washing. However, a study done by Chavarria *et al*., (2004) indicated that washing of asparagus did not have a significant effect in reducing the residue levels of Chlopyrifos in them.

Food processing practices such as blanching, boiling, frying and roasting enhance volatilization and hydrolysis of chemicals hence altering their concentration levels in food (Kiwango *et al.*, 2018). Cooking has been found to reduce deltamethrin residues in vegetables by up to 40% (Tomer *et al.*, 2013). Blanching has been found to reduce residue levels of fat-soluble pesticides by up to 72% and water-soluble by 79% in cauliflower (Sheikh *et al.*,2013). Residue levels of organophosphates in tomatoes, okra, capsicum, eggplant, beans and cauliflower were found to reduce by 52-100% after being boiled (Satpathy *et al.*,2011). In the same study, frying was found to reduce fat-soluble pesticide such as profenofos, endosulfan and bifenthrin by 96.75%, 94.32% and 98.71% respectively. Studies done by Keikothaile *et al.*,(2011), Yang *et* al., (2012), Bajwa *et al.,* (2014) and Inonda *et al.*,(2015) showed that washing, cooking and other handling/processing practices of vegetables reduce pesticide residues upto 50%. Other methods such as blanching and washing vegetables with vinegar were also found to reduce pesticide residues (Wanwimolruk *et al.*, 2015). However, in another study, it was found that pre-heating, pulping, half-pasteurization and evaporation increased the concentration levels of deltamethrin residues by 2.33% while reducing that of endosulfan by 66.5% (Tomer *et al.*,2013).

Various environmental factors such as precipitation, humidity, Air movement (wind), temperature and radiation affect pesticide residues in vegetables in various ways. Precipitation such as rain and hail wash off pesticides from surfaces of vegetables hence reducing the amount of pesticide plants. Excess moisture in plants is excreted through leaves and roots, this also helps in reducing pesticide residues. High humidity in the atmosphere affects volatilization of pesticides by lessening it. Air movement in form of wind physically removes pesticides on the surface of plants. It also influences volatilization of pesticides on the plants. Radiation from sunlight enhances the breakdown of pesticides on plants and increases volatilization of pesticides (Edwards, 1975). High temperature increases the rate pesticide degradation on plant surfaces (Ebeling, 1963).

2.8. Human exposure to pesticides

Pesticides get into human bodies through various exposure routes which include inhalation, dermal contact, ingestion, injection and ocular. Multiple exposures to pesticides can occur through the various routes. For example dermal absorption can occur through accidental contact, occupational exposure, residues on surfaces, contact with contaminated clothing and medicinal use (WHO, 2008). Ingestion of pesticides can occur through accidental ingestion and residues in foods. According to Juraske *et al*., (2009) exposure to pesticide residues through dietary intake is estimated to be five times higher than other exposure routes. Vegetables are consumed raw, steamed or cooked. They are hence more likely to contain pesticide residues compared to other foodstuffs that undergo processing.

Human beings become exposed to pesticide residues through consumption of food. According to Jalalizand *et al*., (2011), pesticide residues contamination levels in vegetables can be reduced by washing. However, removing toxic components from inner tissues is difficult. Therefore, people are at a risk of consuming them. The risk of exposure to pesticides in Kenya has not studied adequately. This needs to be studied to provide information on the risk of exposure through consumption of vegetables. Various studies have been done on the potential negative impacts on health caused by pesticide residues in food. These effects include carcinogenic and teratogenic (Criswell *et al*., 2013). Pesticides have been associated with sarcomas, cancer of the prostrate, pancrease, lungs, ovaries, breasts, kidneys and intestines. They have also been associated with multiple myelomas and brain tumors (Zahm and Ward, 1998; Alavanja *et al.*, 2004).

Pesticides have been found to cause negative effects on the nervous system such as impairing its development which leads to lowered intelligence and behavioural abnormalities (Grandjean and Landrigan, 2006). A study done in California, United States by Roechr (2014) linked severe Autism Spectrum Disorder (ASD) with organophosphates and Pyrethroids before conception and during the third trimester. The study also linked carbamates with delayed development. Some pesticides have also been found to increase the risk of fatal neurological disease known as amyotrophic lateral sclerosis (ALS) which affects the brain and progressively inhibits it to control functions such as movement, speech, eating and breathing. They have also been linked to increased risk of Parkinson's disease (Firestone *et al.* 2005, Hancock *et al.*, 2008).

In addition, pesticides have also been found to cause negative reproductive effects such as stillbirths, low birth weight, early neonatal deaths and spontaneous abortions. They have also been associated with causing hormonal imbalances, reduced sperm count and sterility in males (Goldman, 1997, Grandjean and Landrigan, 2006). Pesticides also weaken the immune system especially in growing children which increases their risk to infectious diseases hence high mortality rates especially in developing countries (Repetto and Baliga, 1996). They can also promote autoimmune diseases such as diabetes, lupus and rheumatoid arthritis and allergy sensitization reactions (Devon, 2016)

2.9. Legal framework

Agenda 21, chapter 19 concerns environmentally sound management of toxic chemicals, including prevention of illegal international traffic in toxic and dangerous products. These include pesticides and other chemicals. Chemicals are important since their use is essential in meeting economic and social goals. However, best practice in their use should be applied to ensure they are used in a cost-effective manner and with high degree of safety. The Agenda sets out six areas on environmentally sound management which include: expanding and accelerating international assessment of chemicals, exchanging information on toxic chemicals and chemical risks, establishing of risk reduction programmes, strengthening national capabilities and capacities for management of chemicals and preventing illegal international traffic in toxic and dangerous products (UNCED, 1992).

World Health Organization together with United Nations Environment Program established Intergovernmental Forum on Chemical safety (IFCS) which convened governments and non-governmental organization and recommended new International treaty to restrict Persistent Organic Pollutants in 1998. This led to signing of formal treaty in Stockholm in 2001. The convention identified twelve organic chemicals of global concern and governments agreed to eliminate them. Among the chemicals identified included eight pesticides which include Aldrin, Chlordane, Dieldrin, Endrin, Heptachlor, Hexachlorobenzene, Mirex and DDT. Other chemicals included polychlorinated biphenyls, dioxins and furans. Kenya has set up Pest Control Products Board since it is a signatory to the treaty. The board's mandate is to regulate and restrict pesticide products that are sold in the local market. However, enforcement has been a challenge as banned products are found in the market through illegal importation.

The Rotterdam Convention came into force in 2004. It focused on exporting hazardous chemicals to developing countries. The treaty requires that any exporting enterprise from signatory country should provide advances notice or a prior informed consent to the importing country when shipping hazardous chemicals listed in the special annex of the convention. The annex contains a list of forty-three substances- eleven industrial chemical and thirty-two pesticides (Rotterdam Convention, 2004).

According to Kone II (2014), despite the existence of these conventions that prohibits transportation and export of toxic and hazardous waste, most developed countries continue to dump them in Africa. Most pesticide producing companies are found in developed countries, there is continual export of banned pesticides from these companies to developing countries. Pesticide Control Act, 2006 of Kenya provides for regulation of importation, exportation, manufacture, distribution and manufacture of products used for the control of pests and of the organic function of paints and animals and for connected purposes. Pest Control Products Board has been mandated to enforce the Act. The Board has listed six restricted pesticides namely Benomyl, Carbonfuran or Thiram, DDT, Ethyl Parathion, Methyl parathion and phosphamidon.

It has also listed thirty one pesticides that are banned for use in Kenya such as Alachlor, Lindane, Aldicarb and Mercury compounds among others (PCPB, 2014)**.**

2.10. Conceptual framework

Pesticide residue level in foods is an important element in food safety issues. Farmers play a major role in determining the concentration of pesticide residues that can be found in fresh produce. Since they are the ones who apply pesticides, they are the ones who are to observe pre-harvest interval before they harvesting produce. Dietary intake pesticide through residues in vegetables is influenced by handling practices. Washing and boiling vegetables before consumption affects the concentration of pesticide residue on vegetables (Inonda *et al*., 2015). Other handling practices such as fermentation, washing with vinegar and storage can affect pesticide residues in foods. Exposure to pesticide residues in vegetables is a function of consumption rate and the concentration of pesticides in the vegetables. Exposure dose is estimated using the weight of a person, concentration of pesticide residue in food and food consumption rate (USEPA, 1996).

Environmental factors such as precipitation, radiation, humidity, air movement and temperature have been found to affect pesticide residues in foods. High environmental temperature and humidity enhances vapour pressure of chemicals and increases degradation of pesticides. This could consequently lead to enhanced toxicity of the chemical pesticides (Viswanathan, 1989, Baetjar, 1968). High environmental temperature especially during the dry season increases the degradation rate of pesticides by photolysis. Precipitation such as rain also affect pesticide residues in foods by action of wash off by rain drops. This therefore reduces the amount of pesticide left on the surface of food such as vegetables and fruits.

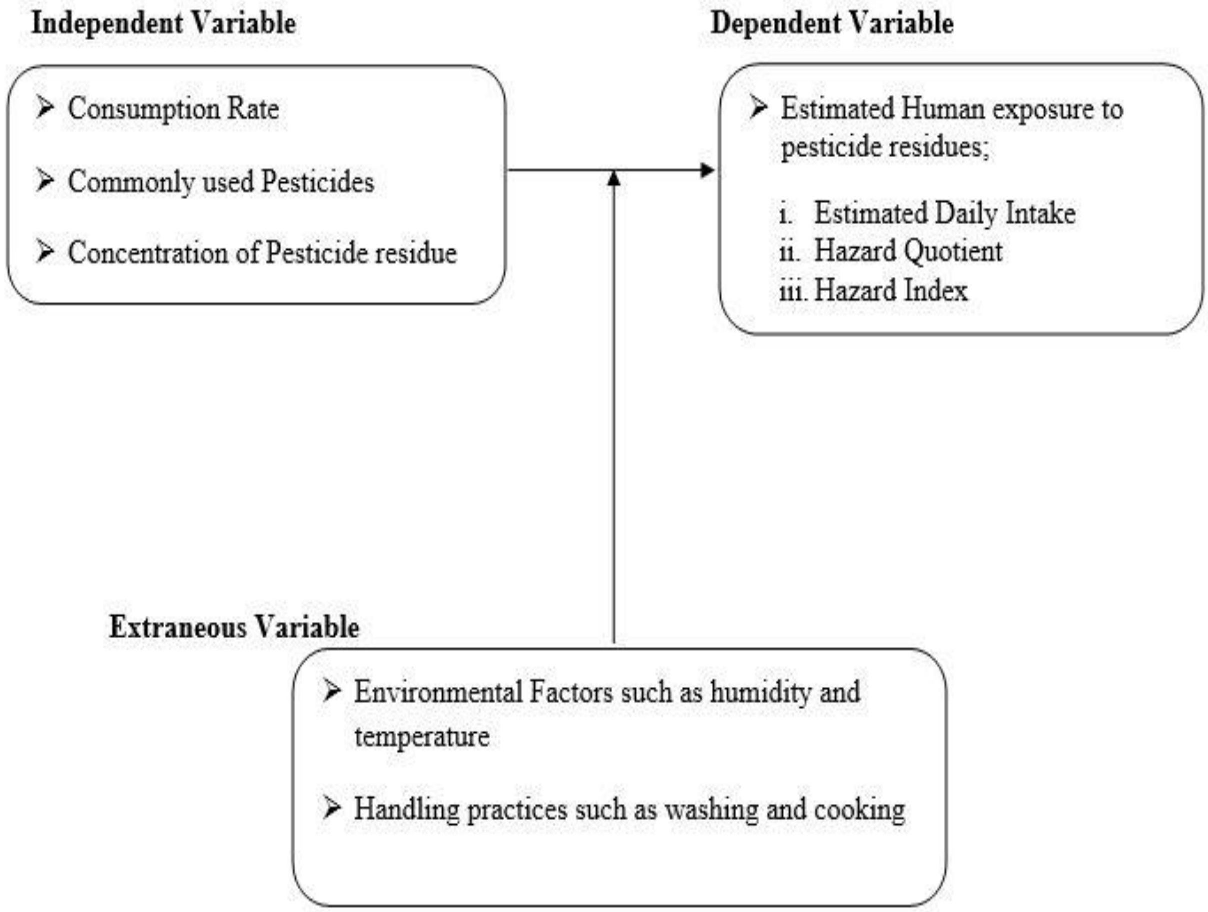

Figure 2.3: Conceptual framework

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

3.1.1 Description of the Study area

Nakuru town lies at a of Latitude: -0.283, Longitude: 36.067 and is 1,850 metres above sea level. It is the headquarters of Nakuru County. It consists of two constituencies which include Nakuru town west and Nakuru town east. It covers an area of approximately 325.30 square kilometres .The town is located 160 kilometres North West of Nairobi city along the east-west rail transport route from Mombasa to Kampala. The town is the fourth largest in Kenya after Nairobi, Mombasa and Kisumu.

The population of Nakuru town is approximately 310,421 (KNBS, 2010). The population of the town is projected to increase due to rural-urban migration. The large population increases demand for agricultural products especially food crops and other resources such as water and land. With increased demand of land for settlement, less land is left for agriculture. On the other hand, more food is required to feed the increasing population; hence farmers are under pressure to produce more and blemish-free food products. This leads to farmers using pesticides to provide such products.

Nakuru town has temperate climatic conditions with the coldest months being between June and August. The hottest month is January. The town has an average annual high temperature of 25.6°C and low of 9.8°C. It has an average annual precipitation of 963mm (World Meteorological Organisation, 2019). Most parts of the town is in ecological zone III which has suitable conditions for agricultural activities.

Economically, agriculture is the backbone of Nakuru town in Nakuru County. However, there are also commerce and industries that are both agricultural and non-agricultural. the agricultural sector provides raw materials for industries which such as flour milling, animal feeds manufacturing and dairy processing plants with some of them being hosted within Nakuru town. Other industries which are non-agricultural include oil refineries, soap manufacturing and Car & General Motorcycle plant. The Central Business District is dominated by commerce while peri-urban areas are mainly dominated by agriculture. Main crops grown in Nakuru County are wheat, barley, maize, beans, vegetables, currently pyrethrum is picking up. The area also hosts various tourism attraction sites such as Hyrax Hills and Lake Nakuru National Park being a bird sanctuary with flamingoes and pelicans among others.

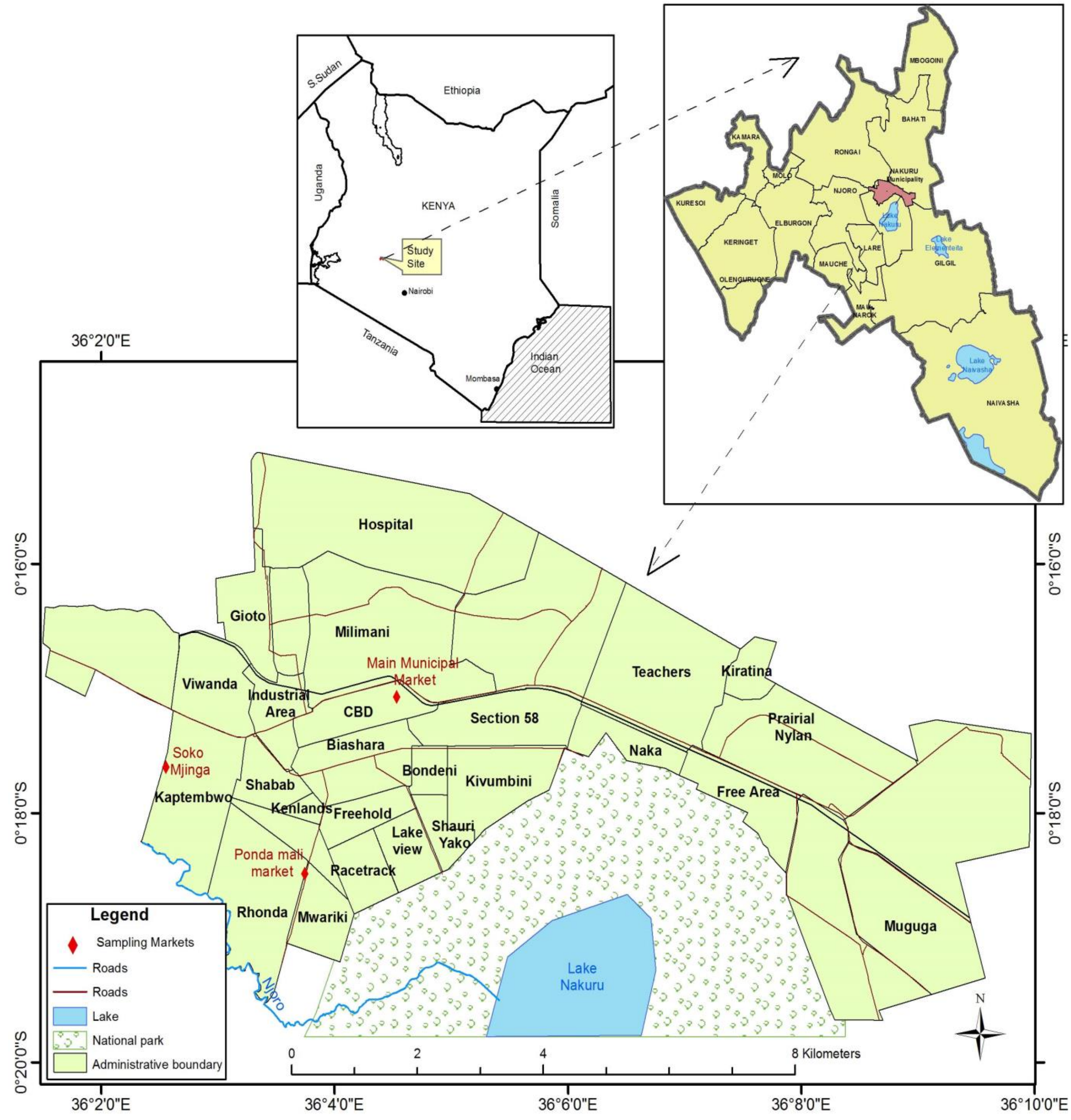

Figure 3.1: Map of Selected Vegetable Markets

Source: KNBS, 2013

3.2 Research Design

This study used cross-sectional study design which included a social survey and laboratory analysis. Structured questionnaires were used for social survey to get data on sources and consumption rates of selected vegetables and most commonly used pesticides on vegetables. Experimental design was used to find out the concentration of pesticide residues in the most consumed vegetables.

3.3 Sample size determination

The sample frame for this study included; markets, farmers, sources (areas of production), key informants, vendors and consumers from the selected markets in Nakuru town.

3.3.1 Markets sample size

Purposive sampling was used in selecting three markets in Nakuru town. This was done considering the size and the population they serve. The markets also play a major role as distribution points of agricultural products. Data was collected from the selected open air markets which included; Soko-Mjinga, Ponda-Mali and Main Municipal Market town market.

3.3.2. Consumers sample size

The formula applicable for sample size calculation for this study was;

$n=z^2pq/d^2$ (equation 3.1) (Bartlett *et al*., 2001). The formula was used since the proportion of the population consuming the selected vegetables within the study area in not known.

Where;

n =sample size,

Z^2 = desired confidence level,

d^2 = desired level of precision,

p = estimated proportion of the population that consume the selected vegetables,
q = estimated proportion of the population that does not consume the selected vegetables,
Z = 95%,

p and q = 0.5

d^2 = 0.05.

Therefore, by substituting the formula with the given figures the sample size for this study was; n = $1.96^2 \times 0.5 \times 0.5 / (0.05)^2$ = 385. Since the total number of consumers who get their vegetables from the selected markets was not known, their estimate number was calculated using the number of vendors in each market. Secondary data from the market supervisor's office and payment offices showed that the approximated number of vendors was 2300, 350 and 310 in Main Municipal Market, Soko-Mjinga and Ponda-Mali market respectively. The number of consumers who buy from each vendor was estimated to be 40 for Main Municipal Market market and 20 for Soko-Mjinga and Ponda-Mali market. This gives a total number of consumers for each of the market to be 92000, 7000 and 6200 in Main Municipal Market, Soko-Mjinga and Ponda-Mali respectively. Consumers sample size of 385 was distributed proportionately. This gave 337, 25 and 23 consumers for Main Municipal Market, Soko-Mjinga and Ponda-Mali markets respectively.

Table 3.1: Number of Vendors, Consumers and Respondents

Market	**Vendors**	**Consumers**	**Research respondents**
Main Municipal	2300	92000	337
Soko Mjinga	350	7000	25
Ponda Mali	310	6200	23
Total			385

3.3.3. Sample size for Key Informants

The key informants were purposively chosen from the Ministry of Agriculture, Livestock and Fisheries and Horticulture, Crops Development Authority (HCDA). Two Crops officers from each of the two entities in Nakuru County headquarters were interviewed. They gave information on the sources of commonly consumed vegetables supplied to Nakuru Town. Other key informants included agricultural extension officers from selected sources. One extension officer was chosen from each selected source bringing the total to three officers. The total number of key informants included in the study was seven.

3.3.4. Sample of Vegetable Sources

Sampling of vegetable sources was done after the key informants had identified the sources. A total of seven sub-counties were identified as sources that supplied vegetables to Nakuru town. They included; Njoro, Bahati, Subukia, Rongai, Molo, Nakuru town west and Nakuru Town east sub-counties.

The formula $nf = n \div \langle 1 + n / N \rangle$ (equation 3.2) according to Araoye, (2004) was used to calculate the sample size.

Where; nf is the desired sample size when the population is less than 10,000.

N is the number of sites (sub-counties)

n is the number of sites intended to be covered

The sample size was calculated as follows:

N=7, n=6, therefore by substituting the figures;

nf = 6/ (1+ (6/7)),

nf = 6/ 1.8571

nf = 3.2308, therefore the sample size was taken to be 3 sources after rounding off the figure.

The specific sources (areas) to be included in the study were selected through simple random sampling by lottery without replacement. The sources that were included in the study were Njoro, Subukia and Rongai sub-counties.

3.3.5. Sampling of farmers

Sampling of farmers was done after the sources of vegetables had been sampled and their number had been known. According to unpublished sources from the sub-counties, the number of vegetable farmers for Njoro, Subukia and Rongai were 1214, 2618 and 928 respectively. The sample size was calculated using the formula $nf = n \div \langle 1 + n / N \rangle$ (equation 3.2) by Araoye, (2004).

N=4760, n=150

nf = 150/(1+ (150/4760))

nf = 150/1.032

nf = 154.8.

The sample was taken to be 155 farmers. They were then distributed proportionately to the sources as 40, 85 and 30 farmers for Njoro, Subukia and Rongai respectively based on vegetable farmer population.

Table 3.2: Number of vegetable farmers distributed in the sub-counties

Sub-Counties	**Total number of farmers in the Sub-Counties**	**Sampled number of farmers in the Sub-Counties**
Njoro	1214	40
Subukia	2618	85
Rongai	928	30
Total		155

3.3.6. Sampling of vendors

Three vendors for each commonly consumed exotic vegetable were randomly selected. Since the study targeted three (3) most consumed exotic, therefore the total number of vendors included in the study were nine (9) from each market. Fresh vegetables were bought from the vendors in the three selected markets.

3.4. Sampling Procedure

3.4.1. Field Survey

In administering questionnaires, multi-stage sampling method was used. Purposive sampling was first used to select markets which include; Main Municipal Market, Ponda mali and Soko mjinga. These markets were used as study clusters. Simple random sampling method was then used to select consumers who participated in the study. Farmers of most consumed vegetables were randomly selected from each sources of production and gave information on commonly used pesticides.

3.4.2. Validity and Reliability

Validity is the degree to which an instrument measures what it intends to measure (Kumar, 2011; Kimberlin and Winterstein, 2008). Two experts from the department of Environmental Science and the department of Crops, Horticulture and Soil (Egerton University) assessed the validity of food frequency questionnaire and farmer's questionnaire. Their responses were incorporated so as to enhance the validity of the tools.

Reliability is the ability of a research tool to produce identical results when used repeatedly under similar conditions (Kumar, 2011). The questionnaires were pre-tested for reliability through a pilot study. The pilot study included 36 consumers who were randomly selected from other markets and 15 farmers from Nakuru Town West sub-county.Computed Cronbach's Alpha value was calculated using internal reliability technique which gave a value of 0.72 for consumers and 0.68 for farmers, hence the tools were considered reliable (Franken and Wallen, 1990).

The High performance liquid chromatography used for pesticide residue analysis used for the study was pretested to ensure that they work properly. Pesticide standards of commonly used pesticides acquired from Sigma-Aldrich, Germany were analysed using High performance liquid chromatography (Waters, 600 controller) to ensure that the machine can detect them. This was done to enhance validity and reliability of the machine to analyse pesticide residues in composite samples of most consumed vegetables.

3.5. Data Collection

3.5.1. Social Survey

Structured questionnaires were used to collect information on consumption rates and commonly used pesticides among consumers and farmers respectively. A standard food frequency questionnaire was administered to randomly selected consumer (> 18 years old) from selected markets to obtain information on individual consumption rates. The information collected from the survey included individual's frequency of consumption of exotic vegetables and amount (kg) consumed per consumption. Estimated consumption rate was calculated from the collected data using the formula: Estimated consumption rate = amount of vegetable consumed in kg/day (USEPA, 1996b). Questionnaires were also administered to key informers to find out sources of vegetables (Appendix II) and to farmers to get information most commonly used pesticides on most consumed exotic vegetables (Appendix V).

3.5.2. Pesticide Residue Analysis

Pesticide residue analysis was done using AOAC 2007.01 method. It was conducted to obtain data on concentration of pesticide residues in most consumed vegetables. The analysis was conducted at a laboratory in University of Eldoret that meets the Codex guidelines on good analytical practice. Handling of samples that is, sampling, packaging, transmission, reception and storage to avoid contamination was done according to the same guidelines. Samples were stored at a temperature range of between $1^{\circ}C$ to $5^{\circ}C$ away from direct sunlight waiting for analysis. Quick, Easy, Cheap, Effective, Rugged and Safe Method commonly known as the QuEChERS was used in the analysis of pesticide residues (Anastassiades *et al.*, 2003). This method is also registered as AOAC 2007.01 official method for pesticide residue analysis

3.5.3. Equipment, Materials and Reagents

The equipment that were used in the laboratory included; HPLC with Reversed-phase column, vortex mixer, blender, chopper (in this case the vegetables were chopped using a knife), centrifuge, liquid dispenser (Calibrated pipet was used), 6 teflon centrifuge tubes, analytical balance, sample microtubes, and micro-syringe.

The reagents that were used during laboratory analysis included; Acetonitrile (MeCN), deionised water, anhydrous Magnesium Sulfate (Reagent grade in powder form), Sodium Chloride (NaCl) (ACS grade), Pesticide standards: Lambda-cyhalothrin and Cypermethrin, Trans-Cypermethrin D6 was used to prepare an internal standard by making a solution of 20 µg/mL in MeCN, Dispersive Solid Phase Extraction (D-SPE) sorbents: Primary Secondary Amine (PSA), Acetone and Dry ice. The stock solution of 1000µg/mL was prepared in the solvent and working standards pesticide mixtures of 50 and 10 µg/mL were prepared in MeCN.

A kilogram of the three most consumed fresh vegetables (Kales, Spinach and tomatoes) were purchased randomly from 3 vendors for each vegetable in the selected markets. They were packed in triplicate for each sample from each of the selected markets and labelled. They were stored below $5^{o}C$ for transportation to laboratory for analysis. In this analysis, only the edible parts of the vegetables such as leaves were used.

3.5.4. Sample preparation

Triplicates of vegetable samples were chopped and shredded in a blender to obtain a homogeneous composite sample. The leaves of kales and spinach were used while for tomatoes, the whole fruit was chopped and blended to get a homogenized sample. 10g of the homogenized sample was weighed into 50mL Teflon centrifuge tube. 10mL of MeCN was added using a dispenser (pipet), and the sample was vigorously shaken for 1min using a Vortex mixer at maximum speed. 4 g anhydrous $MgSO_4$ and 2g NaCl was added and mixed immediately on a Vortex mixer for 1 min. This was done immediately to prevent formation of $MgSO_4$ conglomerates. 40 mL Internal standard solution was added, mixed on a vortex mixer for another 30 s, and extract was centrifuged for approximately 5 min at 5000 rpm. 1 mL aliquot of upper MeCN layer was transfered into 1.5 mL microcentrifuge test tube containing 25 mg PSA sorbent and 150 mg anhydrous $MgSO_4$ and capped. The mixture was then shaken with Vortex mixer for 30 s. Extracts were centrifuged for 1 min at 6000 rpm to separate pellet from the supernatant, and 0.5 mL of extract was transferred to HPLC analysis.

High performance liquid chromatography machine Waters 600 Controller model was used for analysis. The machine was connected to a detector Waters 484 Tunable Absorbance Detector model. Acetonitrile and water were used as mobile phase in a ratio of 80:20 v/v respectively. The column (LiChrospher® 100 Rp-18, 5µm) was at room temperature while the flow rate was 1ml/min. The wavelength of the detector was set at 205nm with a sensitivity of 0.5. 20µL of extract was injected into the HPLC machine for analysis using a micro-syringe.

Plate 3.1: Machine setup; plotter, detector and HPLC machine

Plate 3.2: Extracted samples ready for analysis

Pure standards of cypermethrin and lambda-cyhalothrin were ran in the HPLC machine and the retention time of each was determined. This was done to identify peaks of the two pesticides that were of interest in different samples. Concentration of pesticide residues for each pesticide tested was determined by calculating the area of the peaks. This was done using Motic Images Plus 2.0. Hence; Concentration of pesticide residue = Area of peak

3.5.5. Recovery Assays

Fresh organic vegetables samples (Kales, tomatoes and Spinach) were bought from certified organic farmers. They were used in testing the recovery rates of the pesticides using the QuEChERS method. The samples were first analysed to ascertain that they really had no pesticide residues since the farmers do not use chemical pesticides. The vegetables were spiked with known concentration of pesticide standards. Extraction of pesticide was done using the procedure and analysed to determine the recovery rate. This was done in triplicates for the three types of vegetables. The results of the recovery percentages from the vegetables were as indicated in Table 3.3.

Table 3.3: Mean Recovery rates of pesticides

Vegetables	Cypermethrin (% Mean±SD)	Lambda-Cyhalothrin (% Mean±SD)
Kales	95.7±0.01	91.97±0.019
Tomatoes	87.78±0.014	90.65±0.049
Spinach	97.93±0.006	95.72±0.023

3.6. Human Exposure Assessment

Human exposure assessment is the scientific way of estimating the magnitude, frequency and duration of human exposure to an agent in the environment (USEPA, 2014). There are different approaches that have been suggested in estimating exposure, however, this study used Estimated Daily Intakes (EDI), Hazard Quotient (HQ) and Hazard Index (HI).

Estimated Daily Intake (EDI(mg/kg-bw/day)) was calculated using the formula: $EDI = \langle RPC \times CR \rangle \div Bw$ (equation 3.3) according to USEPA, (1996).

Where; EDI is the Estimated Daily Intake

RPC is Residual pesticide concentration (mg/kg)

CR is Consumption Rate$_{(kg/day)}$

Bw is Average Body Weight. The average body weight of an African adult is 60.7 Kg (Walpole *et al*.,2012)

Hazard Quotient (HQ) is the ratio between Estimated daily intake of a substance and a reference dose (a known level at which no adverse effects are expected in this study, the ADI was used.) (USEPA, 2000).

$HQ = EDI \div ADI$ (equation 3.4)

Where; HQ is the Hazard Quotient

EDI is the Estimated Daily Intake

ADI is the Acceptable Daily Intake

Hazard index (HI) is similar to Hazard quotient for exposure to a single substance. When there is exposure to more than one substance, the Hazard index is calculated by summing up Hazard quotients for exposure to each substance (USEPA, 2000).

Therefore; HI = HQ_1 +HQ_2+…….(equation 3.5)

3.7. Data Analysis

Collected data was analysed using statistical package for social sciences 22. Descriptive statistics were used to organize data collected using questionnaire on consumption of vegetables. Most commonly used pesticides were also arranged using descriptive statistics. Descriptive statistics for concentration of pesticide residue (area of peaks) were calculated using Motic Images plus 2.0. One-way Analysis of Variance was used to determine if there was significant difference in the concentration of the two pesticides in the three types vegetables among the three selected markets. Estimated daily intakes, hazard quotient and hazard indices for the pesticides analysed were calculated for assessing the extent of human exposure and in order to determine if the consumers are at risk of significant health effects. Independent T-test was used to determine if there was significant difference in hazard quotient between average and high consumers. The P-value used for the statistical test was $P<0.05$. Summary of data analysis is given in table 3.4 below.

Table 3.4: Data Analysis Summary

Research Question	Variables	Statistical tools
1. What is the consumption rate of three most consumed exotic vegetables sourced within Nakuru County?	Consumption rate	Descriptive statistics (Mean)
2. What is market chain of three most consumed exotic vegetables sourced within Nakuru County in Nakuru town?	Production sources	Descriptive statistics
3. How are the vegetables handled by consumers before consumption?	Handling practices	Descriptive statistics (Percentages)
4. Which are the most commonly used pesticides and what are their chemical components and properties?	Commonly used chemical pesticides	Descriptive statistics (Percentages)
5. What is the concentration of most commonly used pesticides in vegetables?	Concentration of pesticide residue.	1. Descriptive statistics (Mean) 2. One-way ANOVA test
6. What is the estimated exposure to pesticide residues in from vegetables?	Daily intake Hazard quotient and indices for pesticides.	3. Estimated Daily intakes 4. Hazard Quotient 5. Hazard Index 6. Independent T-test

CHAPTER FOUR

RESULTS AND DISCUSSION

The results discussed in this chapter are in line with the specific objectives of the study. The results represented are from a social survey carried among consumers, farmers and stakeholders. The stakeholders included suppliers and representatives from HCDA.

4.1 Demographics of respondents

The results in this section indicates demographics of consumers and farmers who were among the respondents that participated in the survey.

4.1.1. Demographics of consumers

Sixty six point five percent of the respondents (n=385) were female while the rest were males as shown in Figure 4.1. This is consisted with other studies that suggest women are the principal buyers in households (Githukia *et al*, 2014). In many cultures in Kenya, going to the market is one of the females' roles , hence most respondents in the market were women. The mean age of the respondents was 33.08 $\pm$ 9.226 years with the youngest respondent being 18 years old and the oldest respondent was 60 years old. The highest percentage (44.68%) of respondents were between 27-35 years old hence most of the consumers found in the market were youths. The results concur with Githukia *et al.*, (2014) most buyers in markets are youths.

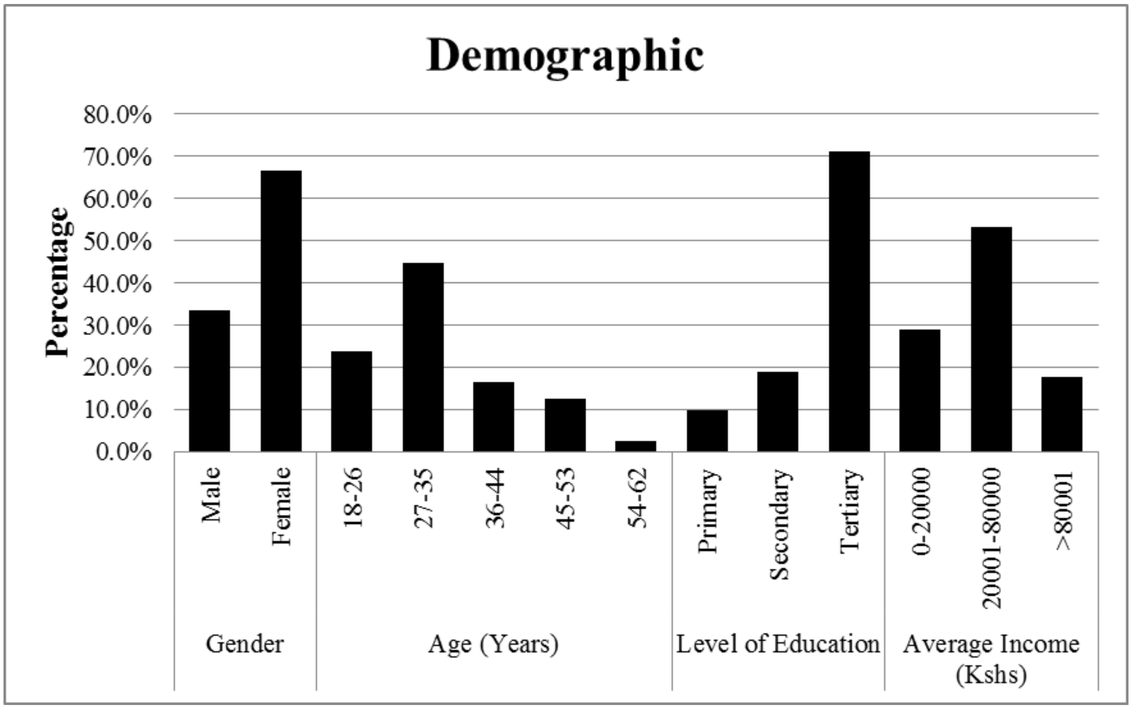

Figure 4.1: Demographics of consumers

All respondents had attended formal schooling with the least level being basic primary schooling. 71.2 % of the study population (n=385) had attained tertiary level of education which included attending college, universities and technical institutions such as polytechnics.

The implementation of Free Primary Education since 2003 has led to increased literacy levels both in the urban and rural areas as more people are able to access primary school of education. According to KIPPRA, 2016, there was increased enrolment in secondary institution and subsequent increase of student enrolment in tertiary institutions. This was attributed to introduction of subsidised secondary day fees and increase in number of tertiary institutions such as technical institutions for craftsmen and technicians and universities.

Estimated household income of the respondents was categorised as low, middle and high income with the low being KShs 0-20000 and high those with more than KShs 80000. In the study, 53.2% (n=385) were from middle income earning households. This is because the study was done in an urban area where most people either have blue or white collar jobs. The findings are in line with other studies that show people in urban areas are more economically empowered than those in rural areas (KIPPRA, 2016).

4.1.2. Demographics of Farmers

Farmers from Subukia, Njoro and Rongai sub-counties were interviewed to provide information on the most commonly used pesticide. The results indicated that the average age of the farmers interviewed was 44.25±10.996 years. Most farmers had atleast lower primary level apart from 16.31% who have never attended school hence they cannot read and write as shown in Figure 4.2. In another study done in Central Rift Valley in Ethiopia, the results indicated that 55% of the farmers were illiterate (Mengistie *et al.*, 2015). Even though most of them farmers in this study were found to be literate, 68.23% responded to not reading the instructions and that they relied on instructions given by agrochemicals sales persons. Education among farmers is encouraged as literate farmers can understand better the effects of pesticide on human health and the environment compared to less literate farmers (Rios-Gonzalez *et al.*,2013).

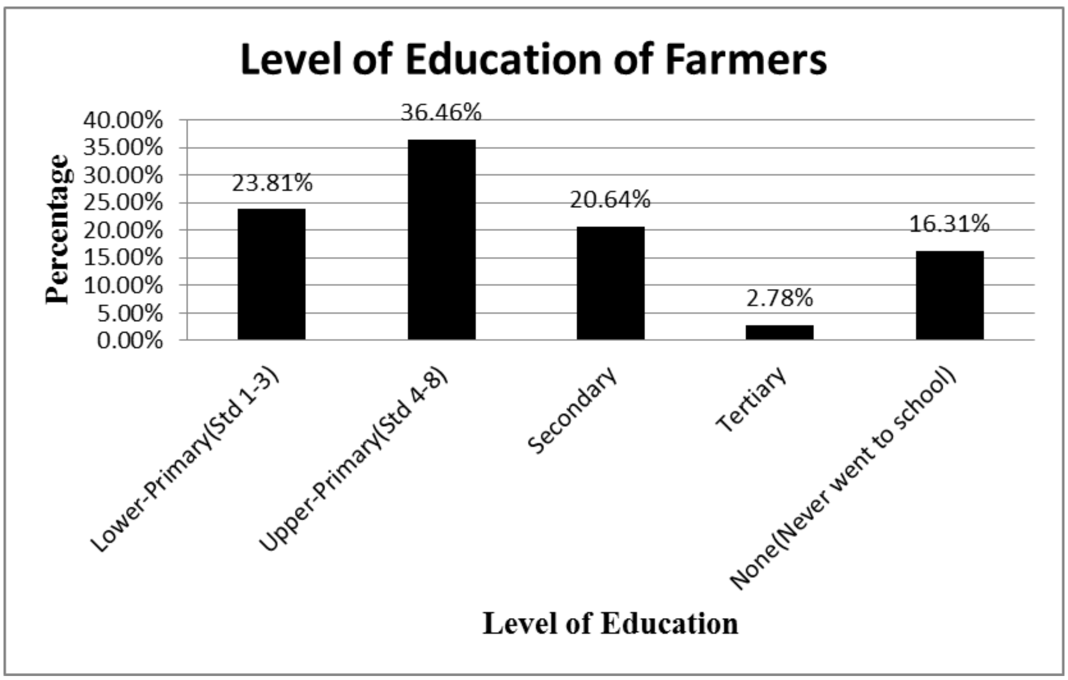

Figure 4.2: Farmer's level of Education

4.2. Consumption of vegetables

The three most commonly consumed vegetables in terms of number of people who consume were Tomatoes, Spinach and Kales. Cabbage and Other vegetables such as lettuce, broccoli and cauliflower were among the least consumed vegetables. Table 4.1 indicates the percentage of people who consume various vegetables.

Table 4.1: Commonly consumed vegetables

Vegetable	Percentage of Consumers (n=385)
Kales (*Brassica oleracea var. acephala*)	91.95%
Spinach (*Beta vulgaris subsp. cicla*)	94.55%
Tomatoes (*Solanum lycopesicum*)	100%
Cabbage	53.51%
Other vegetables	13.25%

In terms of consumption rates, Kales had the highest mean than Tomatoes even though they are consumed by all respondents as indicated in Table 4.2. Most consumers consumed a mixture of vegetables such as mixing Kales, Spinach and tomatoes. The average consumption rate of the most consumed vegetable was 116g/per person/day as indicated in table 5. The results were consisted with other studies that show the three vegetables are most consumed vegetables in Kenya (Maina *et al.,* 2008). Average daily consumption of vegetables is below the WHO recommended intake of vegetables of 300g/per person/per day. A study done in India found out that despite the country being the second largest fruit and vegetable producer globally, vegetable consumption was below the recommended amount (Sachdeva *et al*, 2013). According to Lock *et al*., (2015), in a study on vegetable and fruit consumption among Saudi University students, found out that vegetable consumption among the students was below the recommended amount. Even though the results concur with other studies that indicate low vegetable consumption among men and women, in this study only exotic vegetables were considered. Hence consumption rate of vegetables (both exotic and indigenous) might be higher than the results in the study.

Table 4.2: Consumption rates of selected exotic vegetables

Vegetables	Consumption rate (Mean±SD)$_{(kg/person/day)}$		
Kales	0.116	±	0.111
Tomatoes	0.107	±	0.052
Cabbage	0.075	±	0.085
Spinach	0.078	±	0.086
Others	0.021 ± 0.066		

4.3. Sources of vegetables in Nakuru County.

Agricultural products such as fruits and vegetables come from farms. The products are then distributed to various part of a region for sale. Most fruits and vegetables in Nakuru town come from farms near and far from the town. It being a transit town, it gets it fruits and vegetable supply from various parts of the country. These come from both small scale and large scale farmers who are suppliers. Availability and price of these products are determined by their demand and supply forces in the market. Distance and conditions of road network play a major role in transportation of the products to the market.

The sources were identified to be Njoro, Bahati, Subukia, Rongai, Molo, Nakuru town west and Nakuru Town east sub-counties. Three sources were samples which included; Njoro, Subukia and Rongai sub-counties. Most of these sources are peri-urban and rural agricultural areas. This indicates that the town depends on these areas for vegetables. This showed that urban dwellers depend on rural and peri-urban areas for agricultural goods. Urban areas provide markets for the goods produced in those areas that are in surplus. This also helps develop business and a source of income to farmers and their families in production areas.

4.4. Commonly used pesticide and concentration of residues in vegetables

4.4.1. Commonly used pesticides on vegetables

All farmers responded that they used pesticides on kales and tomatoes. However, none used pesticides on spinach. According to the farmers, they did not spray pesticides on spinach because it is rarely attacked by pests and diseases. Farmers named a total of thirty three pesticides that they used according to their trade names. The pesticides named by the farmers were classified according to active ingredient. The most common pesticides used in the selected vegetables used by farmers included Cypermethrin (21.21%), Lambda-cyhalothrin (18.18%), Alpha-cypermethrin (15.15%) and Mancozeb (12.12%) as indicated in table 6. Others included Dimethoate, Flubendiamide, Pyrimethanil, Sulphur, Emamectin benzoate, Chlopyrifos, Thiophanate, Imidacloprid and Copper hydroxide as indicated in Table 4.3. This indicates that synthetic pyrethroids were being used more than organophosphates on vegetables. Carbamates and organochlorines are rarely used. In a study done in Nairobi on pesticide use in 2015 showed that organophosphates were used more than synthetic pyrethroids (Inonda *et al.*, 2015). The same study also indicated that dimethoate was being applied on vegetables.

Even though Dimethoate has been banned for use in vegetables in Kenya since 2012 (MoA, 2012), it is evident that it is still in use. The product is sold under different trade names in the market which makes it difficult for law enforcers to identify them. Farmers on the other hand depend on information provided by sales persons from agro-chemical industries or stores and different pesticide companies on which pesticides to apply. This creates a gap of knowledge among farmers since the business people are interested in making profits.

Table 4.3: Pesticides used by farmers on vegetables and their classification

Active ingredient	Trade name	Type of pesticide	Percentage of farmers using	WHO classification	Vegetables
Alpha-cypermethrin	Alberze, Alfacypermethrin, Bestox, Cyrux, tataalfa	Synthetic pyrethroid	15.15%	II	Kales
Cypermethrin	Cyclone	Synthetic pyrethroid	21.21%	II	Kales and tomatoes
Flubendiamide	Belt	Organofluorine	3.03%	O	Tomatoes
Dimethoate	Danadim, Dimethoate.	Organophosphate	6.06%	II	Tomatoes
Mancozeb	Dithane, Milthane, Mistress, Ridomil, Victory.	Dithiocarbamate	12.12%	U	Tomatoes
Lambda-cyhalothrin	Duduthrin, Karate, kungfu, lambdacylothrin, Vendex.	Synthetic pyrethroid	18.18%	II	Kales and Tomatoes
Pyrimethanil	Methan	Fungicide	3.03%	III	Tomatoes
Sulphur	Palm	Elemental Chemical	3.03%	III	Tomatoes
Emamectin Benzoate	Prove	Insecticide	3.03%	O	Tomatoes

Chlorpyrifos	Pyrinex, Cyclone	Organophosphate	6.06%	II	Kales and Tomatoes
Thiophanate	Thiophate	Carbamate	3.03%	O	Tomatoes
Imidacloprid	Thunder	Neonicotinoid	3.03%	II	Kales and Tomatoes
Copper hydroxide	Copper	Insecticide	3.03%	II	Tomatoes

World Health organisation classifies pesticides in different categories based on toxicity of the technical compound and on its formulations. The classification distinguishes between the more and less hazardous forms of pesticides based on oral and dermal acute exposures. The classification include; Ia=Extremely hazardous, Ib=Highly hazardous, II=Moderately hazardous, III=Slightly hazardous, U=Unlikely to present acute hazard in normal use, FM=Fumigant, not classified and O=Obsolete as pesticide, not classified (WHO, 2009). The pesticides active ingredient used by farmers are classified as shown above (Table 4.3). According to the classification, most pesticides used by farmers in growing vegetables are moderately and slightly hazardous. 72.72% farmers who participated in the study indicated that they used moderately hazardous pesticide as shown in Table 4.4.

Table 4.4: Percentage of farmers using pesticides according to WHO classification

WHO Classification	**Percentage of farmers using**
Ia	0%
Ib	0%
II	72.72%
III	9.09%
U	12.12%
FM	0%
O	6.06%

Exposure to these pesticide can lead to various negative health effects in human beings. People exposed excess doses of Mancozeb are likely to suffer from cancer and goitre since it interferes with iodine intake in the body. Exposure to flubendiamide can cause body tremors, abnormal gait, decreased muscle tone, hypothermia, decreased locomotor activity, exophthalmos with haemorrhage and mild microcytic anemia (Acero, 2017). Dimethoate affects the nervous, muscular, ocular, integumentary, respiratory and digestive systems (Simmons, 2017). Exposure to high doses of mancozeb causes toxic epidermal necrolysis, dyshidrotic eczema, neural tube defects (Nordby *et al.*, 2005) and thyroid disease (Goldner *et al*., 2010).

Pyrimethanil causes histopathological changes in liver and thyroid of humans if exposed for a long period (EFSA, 2006). Emamectin Benzoate has been found to cause damage to the nervous system and liver cells in human beings (Zhang *et al*., 2017). Exposure to chlorpyrifos affects respiratory system, cardiovascular system and central nervous system (United farmers co-operative company limited, 2005). Imidacloprid can impair liver and kidney functions when one gets exposed to it (Arfat *et al*., 2014). Exposure to multiple pesticides can result to synergistic, additive or antagonistic effects. In a study done by (Cedergreen, 2014) found out that synergistic effects were observed in test organism exposed to a combination of pesticides that caused cholinesterase inhibition. The results of the study also indicated that synergism was also observed in test organisms exposed to a combination of azole fungicides. In another study done by Mozo, (2012) indicated that exposure to multiple pesticides that cause endocrine disruption such as pyrethroids (Brander *et al.*, 2016) produced synergistic effects.

4.4.2. Frequency of pesticide application on vegetables

Farmers also provided data on pesticide application practice. They gave information on how often they sprayed vegetables with pesticides. The results indicated that most farmers were not adhering to the required pre-harvest interval period after spraying pesticide and before harvesting their vegetables. 32.23% of the farmers applied pesticides on their vegetables 7 days from the last application. On the other hand, 11.21% of the farmers responded that they applied pesticide on vegetables when they see pest(s) or disease on vegetables as indicated in Table 4.5. Different pesticides have varying pre-harvest interval that is usually indicated in the information sheet bought together with the pesticide. However, since most of the farmers indicted that they do not read instructions given, they therefore fail to adhere to the pre-harvest interval before harvesting their produce. Studies done by Mengistie *et al*., (2015) and Jallow *et al*., (2017) indicated that most farmers were illiterate hence could not read and understand the instructions written on the information sheets of the pesticides.

The farmers also indicated that frequency of spraying pesticides are also influenced by climatic conditions, that is, wet and dry seasons. More pesticide is sprayed during the dry season because more pests appear during that period than during the wet season. The farmers indicated that they harvest the vegetables before spraying. A study done in Central Rift Valley of Ethiopia indicated that farmers applied pesticides more often than required. Frequency of application was also influenced by climatic conditions such as wet and dry season (Mengistie *et* al., 2015). Frequent application of pesticides on vegetables and non-adherence to pre-harvest interval period leads to accumulation of pesticide residues on vegetables.

Table 4.5: Frequency of Pesticide Application

Days	**Percentage of Farmers**
4	11.21%
5	25.91%
7	32.23%
14	18.64%
When pest or disease is spotted by the farmer	12.01%

4.4.3. Concentration of pesticide residues in vegetables

Pure standard samples of cypermethrin, lambda-cyhalothrin and Trans-cypermethrin D6 were dissolved in Acetonitrile and ran individually in the HPLC to determine their retention time. This also helped in identification of the compounds individually. The standards were then mixed to check if the HPLC machine was capable of separating the peaks. Dilution of the standards of 0.0001, 0.001 and 0.01mg/l were used to make calibration curves. Concentration of pesticide residues were determined by calculating the area of peaks that were plotted. This was done using a software known as Motic Images Plus 2.0.

Analysis of concentration of pesticide residues in the selected vegetables was done on the edible parts. This included leaves of Spinach and Kales while for tomato the whole fruit was used. The concentration of the pesticide residues for cypermethrin and lambda-cyhalothrin are as shown in Table 4.6. The results includes pesticide residue concentration according to the markets where the vegetables were purchased. It should be noted that the vegetables were analysed as they were bought from the market and not subjected to any treatment such as washing.

Table 4.6: Concentration of Cypermethrin and Lambda-Cyhalothrin residues

Markets	Vegetables	Cypermethrin (Mean±SD)mg/kg	Lambda-cyhalothrin (Mean±SD)mg/kg
Soko-Mjinga	Kales	1.397±0.478	0.262±0.108
Ponda-Mali	Kales	0.982±0.865	0.341±0.164
Main Municipal Market	Kales	0.238±0.132	0.427±0.219
Soko-Mjinga	Spinach	2.458±0.298	0.352±0.193
Ponda-Mali	Spinach	2.495±0.109	0.24±0.045
Main Municipal Market	Spinach	1.462±0.239	0.365 ±0.28
Soko-Mjinga	Tomatoes	0.232±0.085	0.081±0.037
Ponda-Mali	Tomatoes	0.296±0.076	0.049±0.046
Main Municipal Market	Tomatoes	0.401±0.052	0.119±0.052

Generally, the results indicated that the concentration of cypermethrin residues was higher in all the vegetables than lambda-cyhalothrin. The concentration of cypermethrin in all composite vegetable samples ranged between 0.232 and 2.495 mg/kg with a mean of 1.107±0.912 mg/kg. On the other hand, the concentration of lambda-cyhalothrin ranged between 0.046 and 0.352mg/kg with a mean of 0.216±0.11 mg/kg. This might be because farmers may not be using Lambda-cyhalothrin more often as Cypermethrin. The low residue concentration of lambda-cyhalothrin can also be attributed to its shorter half-life compared to Cypermethrin.

It was also noted that Spinach had the highest concentration of pesticide residues among all the vegetables. The concentration of pesticide residues in Spinach ranged between 1.462 and 2.495 mg/kg for cypermethrin and between 0.352 and 0.24 mg/kg for lambda-cyhalothrin. On the other hand, tomatoes had the lowest residues of both pesticides which ranged between 0.232 and 0.401

mg/kg for cypermethrin and between 0.049 and 0.119 mg/kg for lambda-cyhalothrin. A study done in Egypt to determine the concentration of lambda-cyhalothrin in tomatoes indicated residue concentration ranged between 0.035 and 0.135mg/kg (Malhat *et al.,* 2016).

Although all farmers said they did not spray pesticides on Spinach, it was noted that most of the farms visited had inter-cropped Spinach with Kales. Therefore, the residues on Spinach may have happened accidentally as the Kales are sprayed. In addition, Kales are taller with smooth and shiny surface which make the leaves more exposed to the sun hence photodegradation of pesticides occurs faster. The characteristics of Kales leaves also make it easy for pesticides to wash off when rain falls on them. On the other hand, Spinach plants are shorter with dull and wrinkled leaves, making it difficult to wash off pesticide even if it rains and photodegradation is slower than on Kales leaves.

The maximum residue levels for Cypermethrin in Kales, Spinach and tomatoes are 1mg/kg, 2mg/kg and 0.2mg/kg respectively (FAO/WHO, 2009). Kales from Soko-Mjinga (1.397±0.478mg/kg), Spinach from Soko-Mjinga (2.458±0.298mg/kg) and Ponda-Mali (2.495±0.609mg/kg) and tomatoes from all the three markets exceeded the recommended level. Spinach from the two markets were 1.229 and 1.248 times higher than the recommended level respectively. A study done in Baghladesh indicated that Cypermethrin was below the recommended level in tomatoes (Hossain *et al.,* 2015). The results in this study indicates that consumers who get the commodities that exceed the recommended residue limits from the respective markets are likely to suffer negative health effects such as uncoordinated movements, body tremors and other neurological effects. In addition, consumers who buy vegetables that are not properly cleaned and do not clean and wash them well are also likely to be exposed to the residues. One-way ANOVA test indicated that there was no significant difference in the concentration of Cypermethrin in the three vegetables among the three markets ($F=1.366$, $p<0.05$).

The recommended residues levels for Lambda-Cyhalothrin is 0.3mg/kg in the vegetables (FAO/WHO, 2009). Kales from Ponda-Mali (0.341±0.164mg/kg) and Spinach from Soko-Mjinga (0.352±0.193mg/kg) exceeded the recommended levels. This shows that consumers are likely to suffer negative chronic health effects due to exposure to the pesticide. Tomatoes from

Soko-Mjinga and Ponda-Mali had the least residue levels of 0.081±0.037 and 0.046±0.049 respectively. Therefore consumers who buy tomatoes from these markets are less likely to suffer negative health effects from exposure of the pesticide. However, if they are washed with clean running water and processed as required, exposure to the pesticide residues can be reduced. There was no significant difference in the concentration of Lambda-Cyhalothrin in the three vegetables among the three markets ($F=1.243$, $p<0.05$).

4.5. Handling practices of vegetables

Handling practices of vegetables before consumption were assessed among consumers. The practices included; buying "washed and chopped, no washing again before cooking", "washed and chopped, wash again before cooking", "not chopped, wash and chop before cooking" and "not chopped, wash, chop and eat raw as salad". The results in Table 4.7 indicated that most consumers preferred Kales and Spinach that are already washed and chopped, however, they don't wash again before cooking. All other vegetables were preferred when not chopped and the consumers would wash and chop before cooking them.

Even though this study did not assess the effect of the handling practices practiced by consumers, it is clear that residue concentration of the pesticides can be reduced by handling practices that have been assessed in other studies. These practices include peeling, blanching, cooking, washing with clean running water, washing in vinegar (Keikothaile *et al.*,(2010), Yang *et* al., (2012), Bajwa *et al.,* (2014) and Inonda *et al.*,(2015)). It was observed that most vendors do not wash vegetables as required. They hold the vegetables in a bunch and deep them in a bucket of water. Most of them also recycle the water several times before changing it. Since the vegetables are not properly cleaned as required, the consumers are likely to be exposed to pesticide residues. Therefore looking at the results from table 4.6 and table 4.7, most consumers are likely to be exposed to high pesticide residues from spinach. On the other hand, consumers who buy already washed and chopped vegetables and consume them raw as salad without washing them again are also likely to be exposed more to pesticide residues than other consumers.

Most consumers indicated that they ate other vegetables such as lettuce, cauliflower and broccoli and cabbage raw as salad. These vegetables are therefore more likely to be contaminated with pesticides during consumption as they are not exposed to high temperature through cooking to reduce pesticide residues. Therefore, consumers who eat raw vegetables as salad should ensure that they clean the vegetables properly with clean running water to reduce pesticide residues as exposure can occur when they are not well cleaned before consumption (Krol *et al.*,2000, Wen *et al.,* 2003, Balinova *et al.*,2006). Most consumers responded that they ensured they cooked the three most consumed vegetables, hence they are less exposed to the pesticide.

Table 4.7: Percentage of Handling Practices of Vegetables

Handling Practices	**Vegetables**				
	Kales	**Tomatoes**	**Spinach**	**Cabbage**	**Others**
Washed and chopped, no washing again before cooking	45.2%	0.0%	43.7%	35.6%	0.0%
Washed and chopped, wash again before cooking	15.7%	0.0%	14.3%	7.4%	0.0%
Not chopped, wash and chop before cooking	39.0%	92.5%	42.0%	51.0%	82.0%
Not chopped, wash, chop and eat raw as salad	0.0%	7.5%	0.0%	6.0%	18.0%

Generally, application practices of pesticide by farmers and adherence to pre-harvest interval period plays a major role in determining the amount of residues found in vegetables. If farmers could spray pesticide on vegetables and adhere to the recommended pre-harvest interval, then there could be less residues. On the other hand, handling practices of vegetables by consumers before consumption also affects the amount of pesticide residues in them. Consumers are therefore advised to use methods such as washing vegetables with clean running water, vinegar and properly cooking them to minimize exposure.

4.6. Human Exposure to pesticide residues

Human exposure to pesticide residues is done through health risk assessment by calculating Estimated Daily Intakes, Hazard Quotient and Hazard Index. Estimated Daily Intakes were compared to the Acceptable Daily Intake levels recommended by WHO for the two pesticides.

Hazard Quotients for each pesticide and Hazard Index were also calculated and compared to the recommended value.

4.6.1. Estimated Daily Intake of Cypermethrin

Estimated Daily Intake of Cypermethrin were calculated for consumers of the three most consumed types of vegetables. Table 4.8 represents the results EDI of cypermethrin for average and high consumers. The results indicate that mean estimated intake of Cypermethrin for average and high consumers were 0.0028mg/kg bw and 0.0066mg/kg bw respectively. The largest contribution to the means of average consumers (0.0082mg/kg bw) and high consumers (0.0217mg/kg bw) were from Spinach from Ponda-Mali and Main Municipal markets respectively. The lowest estimated daily intake of Cypermethrin in average consumers was 0.0004mg/kg bw and high consumers was 0.0007mg/kg bw from tomatoes in Soko-Mjinga market. On the other hand, the highest estimated daily intakes in average consumers was 0.0082mg/kg bw and high consumers 0.0217mg/kg bw from Spinach in Ponda-Mali and Main Municipal markets respectively.

The means of estimated daily intake of Cypermethrin for average and high consumers were within the recommended limit of 0- 0.02mg/kg bw (WHO, 2009). The estimated daily intakes of both average and high consumers of all the vegetable in all markets were within the recommended limits except for Spinach from the main municipal market. High consumers of Spinach from the main municipal market had an estimated daily intake of 0.02168mg/kg bw which was slightly higher than the recommended limit. In a study done by Zhi-heng *et al.*, (2010) in Zhejiang Province, China found out that levels of Cypermethrin in vegetables were high than the acceptable limits.

Table 4.8: Estimated Daily Intake of Cypermethrin for Average and High Consumers

Market	Vegetable	EDI_Cyper_Average Consumers$_{(mg/kg\ bw)}$	EDI_Cyper_High Consumer$_{(mg/kg\ bw)}$
Soko-Mjinga	Kales	0.0024	0.0045
Soko-Mjinga	Tomatoes	0.0004	0.0007
Soko-Mjinga	Spinach	0.0029	0.0056
Ponda-Mali	Kales	0.0017	0.0032
Ponda-Mali	Tomatoes	0.0005	0.0027
Ponda-Mali	Spinach	0.0082	0.0137
Main Municipal Market	Kales	0.0005	0.0038
Main Municipal Market	Tomatoes	0.0007	0.0037
Main Municipal Market	Spinach	0.0060	0.0217

4.6.2. Estimated Daily Intake of Lambda-Cyhalothrin

Estimated daily intake of lambda-cyhalothrin was calculated for average and high as represented in Table 4.9. The results are for the three most consumed types of vegetables. The mean estimated daily intake for average and high consumers was computed to be 0.0005mg/kg of body weight and 0.0015mg/kg of body weight respectively. The highest contribution to the means for average (0.0011mg/kg bw) and high (0.0042mg/kg bw) consumers were from Spinach from Main Municipal market.

Acceptable daily intake for Lambda-cyhalothrin recommended by World Health Organisation is 0-0.02mg/kg of body weight (WHO, 2009). The lowest estimated daily intakes among average (8.185E-05mg/kg bw) and high (0.0002mg/kg bw) consumers were for tomatoes from Ponda-Mali and Soko-Mjinga markets respectively. On the other hand, the highest estimated daily intake for average (0.0011mg/kg bw) and high (0.0042mg/kg bw) consumers were for Spinach from main Municipal market. All the estimated daily intakes computed for average and high consumers were within the recommended limit. This might be because residue concentrations of the pesticide were much lower in the vegetables. Since the estimated daily intakes are within the recommended limit, consumers are less likely to experience negative health impact that are associated with the pesticide. A study in Egypt by Malhat *et al.*,(2016) indicated that there were lower dietary intakes of Lambda-cyhalothrin in tomatoes.

Table 4.9: Estimated Daily Intake of Lambda-Cyhalothrin for Average and High Consumers

Market	Vegetable	EDI_Cyhal_Average Consumers$_{(mg/kg\ bw)}$	EDI_Cyhal_High Consumers $_{(mg/kg\ bw)}$
Soko-Mjinga	Kales	0.0004	0.0008
Soko-Mjinga	Tomatoes	0.0001	0.0002
Soko-Mjinga	Spinach	0.0004	0.0008
Ponda-Mali	Kales	0.0006	0.0011
Ponda-Mali	Tomatoes	8.185E-05	0.0004
Ponda-Mali	Spinach	0.0008	0.0013
Main Municipal Market	Kales	0.0004	0.0035
Main Municipal Market	Tomatoes	0.0002	0.0011
Main Municipal Market	Spinach	0.0011	0.0042

4.6.3. Hazard Quotient for Cypermethrin

Hazard quotient is scientific method used to assess exposure to a chemical substance. Hazard Quotient of above one (1) indicate that a consumer is likely to suffer long-term negative health effects due to exposure of the chemical substance being assessed. On the other hand a Hazard Quotient of below or equal to one (1) are less likely to suffer long-term health effects due to the chemical substance. Hazard Quotient (HQ) is the ratio between estimated daily intake of a substance and a reference dose (ADI for Cypermethrin and Lambda-cyhalothrin.). The Acceptable Daily Intake of the two pesticides was established to be 0-0.02mg/kg of body weight per day. This value was set based on No Observable Adverse Effect Level (NOAEL) of 2mg/kg of body weight per day and a 100-fold coefficient of safety factor (USEPA, 2000).

Hazard Quotient of Cypermethrin for average and high consumers were computed and presented in Table 4.10. The mean HQ for high consumers was 0.33 which is 2.57 times higher than that of average consumers of 0.13. The lowest and the highest HQ values calculated for average consumers was 0.02 and 0.41 respectively. On the other hand, the lowest and the highest values computed for high consumers was 0.04 and 1.08 respectively.

Most computed HQ values were less than one except for HQ for high consumers of Spinach from Main Municipal market with a value of 1.08. A HQ value of more than one indicated that the consumers are more likely to suffer negative health effects associated with dietary intake of Cypermethrin. It was also noted that both consumers of Spinach from Ponda-Mali market had significantly high HQ values which can pose a risk of additive effects from other dietary source of Cypermethrin. The results are similar to other hazard quotient values on Cypermethrin in vegetables computed by Chen *et al.*,(2011) whose computation indicated that the values were less than one.

Table 4.10: Hazard Quotient of Cypermethrin for Average and High Consumers

Market	Vegetable	HQ_Cyper_Average Consumers	HQ_Cyper_High Consumers
Soko-Mjinga	Kales	0.12	0.22
Soko-Mjinga	Tomatoes	0.02	0.04
Soko-Mjinga	Spinach	0.14	0.28
Ponda-Mali	Kales	0.08	0.16
Ponda-Mali	Tomatoes	0.03	0.14
Ponda-Mali	Spinach	0.41	0.68
Main Municipal Market	Kales	0.02	0.19
Main Municipal Market	Tomatoes	0.04	0.19
Main Municipal Market	Spinach	0.3	1.08

The negative health effects associated with dietary intakes of Cypermethrin above the recommended value include increased weight of kidneys and liver and adverse changes in to liver tissues. It has also been established to cause pathological changes in the cortex of thymus, liver, adrenal glands, lungs and skin. It may also cause adverse effect to the central nervous system (Occupational Health Services, 1993). The United States Environment Protection Agency classified Cypermethrin as a weak possible carcinogen as it was found to cause benign lung tumors in test organisms (USEPA, 1989).

4.6.4. Hazard Quotient for Lambda-Cyhalothrin

Table 4.11 presents results of computed hazard quotients of lambda-cyhalothrin for average and high consumers. The mean HQ for average consumers was 0.024, this was 3.19 times less than that of high consumers of 0.075. The highest and the lowest HQ values for high consumers were 0.208 and 0.012 respectively. On the other hand, the highest and the lowest HQ values for average consumers were 0.057 and 0.007 respectively. The high HQ values for both consumers contributed to the mean HQ values for the two groups of consumers.

Hazard quotient values of more than one means that consumers are likely to suffer chronic negative health effects associated with lambda-cyhalothrin such as disruption to the nervous system (WHO, 2009). However, the results indicate that, all the calculated HQ values for both average and high consumers were significantly less than one. This means that both consumers are less likely to suffer from adverse negative health effects associated with excess dietary intake of Lambda-cyhalothrin. These lower hazard quotients indicated in the table might be because of the low residue concentration of lambda-cyhalothrin in the vegetables. These results are similar to another study done by Malhat *et al.*, (2016) whose results indicated that there hazard quotient of the pesticide was less than one.

Table 4.11: Hazard Quotient of Lambda-Cyhalothrin for Average and High Consumers

Market	Vegetable	HQ_Cyhal_Average Consumers	HQ_Cyhal_High Consumers
Soko-Mjinga	Kales	0.022	0.042
Soko-Mjinga	Tomatoes	0.007	0.012
Soko-Mjinga	Spinach	0.021	0.040
Ponda-Mali	Kales	0.029	0.055
Ponda-Mali	Tomatoes	0.004	0.021
Ponda-Mali	Spinach	0.039	0.066
Main Municipal Market	Kales	0.021	0.176
Main Municipal Market	Tomatoes	0.01	0.055
Main Municipal Market	Spinach	0.057	0.208

The hazard quotients of Cypermethrin and Lambda-cyhalothrin were compared and the results indicated that there was significant difference in HQ values for both average and high consumers. The HQ values of Cypermethrin were found to be significantly ($t=1.86$, $p<0.05$) higher compared to those of Lambda-cyhalothrin. This result was expected since the concentration of Cypermethrin residues in vegetables were significantly higher than Lambda-cyhalothrin residues. Since the acceptable daily intake of the two pesticides are set to be 0-0.02mg/kg of body weight, high consumers of Spinach from the main municipal market surpassed this limit. This therefore means that they are likely to suffer the negative health effects discussed earlier due to exposure to the pesticide.

4.6.5. Hazard Index

Hazard index (HI) is similar to Hazard quotient for exposure to a single substance. However, when there is exposure to multiple substances, the Hazard index is calculated by summing up Hazard quotients of each substance (USEPA, 2000). In this case the hazard quotients of Cypermethrin and Lambda-cyhalothrin were added for the average and high consumers.

Therefore; $HI = HQ_{\text{cypermethrin}} + HQ_{\text{Lambda-cyhalothrin}}$

A hazard index of less than or equal to one (HI≤1) indicate that the consumer is less likely to suffer chronic negative health effect due to exposure to the assessed substance. On the other hand, a hazard index value of more than one (HI>1) indicates that the consumer is more likely to suffer chronic negative health effects as a result of exposure to the substances being assessed (USEPA, 2000).

The mean hazard index for average and high consumers presented in Table 4.12 were 0.15 and 0.41 respectively. The lowest and the highest HI values for average consumers were 0.03 and 0.45 while that of high consumers was 0.05 and 1.29. The high HI values of average (0.45) and high (1.29) consumers contributed more to their respective means. The finding in the table indicate that the average consumers of Spinach from Ponda-Mali market had the highest aggregate exposure to Cypermethrin and Lambda-cyhalothrin. This is also the same for high consumers of Spinach from Main Municipal market. On the other hand, average and high consumers of tomatoes from Soko-Mjinga market had the lowest aggregate exposure to the two pesticides.

In respect to the three types of vegetables, cumulative pesticide residue decreased in the order; Spinach ≥ Kales ≥ Tomatoes. This means that consumers of Spinach had the highest cumulative exposure to Cypermethrin and Lambda-cyhalothrin while those who consume tomatoes had the lowest cumulative exposure to the two pesticides. Consumers of Spinach from Ponda-Mali and main municipal market had the highest exposure to Cypermethrin and Lambda-cyhalothrin. Their cumulative hazard indexes are more than one which indicates that they are likely to suffer long-term negative health effects due to exposure to the two pesticides. Consumers of tomatoes from Soko-Mjinga market had the lowest cumulative exposure to the two pesticides.

Table 4.12: Hazard Index for Average and High Consumers

Market	Vegetable	HI_Average Consumers	HI_High Consumers
Soko-Mjinga	Kales	0.14	0.27
Soko-Mjinga	Tomatoes	0.03	0.05
Soko-Mjinga	Spinach	0.16	0.32
Ponda-Mali	Kales	0.11	0.21
Ponda-Mali	Tomatoes	0.03	0.16
Ponda-Mali	Spinach	0.45	0.75
Main Municipal Market	Kales	0.04	0.37
Main Municipal Market	Tomatoes	0.05	0.24
Main Municipal Market	Spinach	0.36	1.29

Tomatoes produced the lowest exposure Cypermethrin and Lambda-cyhalothrin. Table 4.12 reveal that total concentration of the pesticides in samples of tomatoes were lower compared to samples pesticide residues concentration of in the other two vegetable types in the market. The results are similar to a study by Chen *et al*., 2011 that indicated lower Cypermethrin and lambda-cyhalothrin levels in tomatoes as compared to other vegetables like Spinach.

The highest hazard index computed for high consumers was 1.2914 as shown in table 4.12. This value compare with the recommended hazard index (≤ 1), it is slightly higher. This indicates that negative health effects associated with the two pesticides are expected among consumers of the related vegetable. On the other hand, the highest hazard index computed for average consumers was 0.4483 which is lower than the recommended value, hence, consumer of the related vegetable are not expected to suffer negative health effects associated with the two pesticides.

The hazard index calculate for this results does not necessarily indicate the true level of exposure to pesticides through residues in commodities since only three types of vegetables have been considered for this study. This does not also indicate total exposure to pesticides through consumption as only two pesticides were studied. This would therefore result to being an underestimate of total exposure to the two pesticides and other pesticides

Table 4.13 below represents results of how much percentage each pesticide residue in the three vegetables contributes to the hazard index.

Table 4.13: Contribution of Lambda-Cyhalothrin and Cypermethrin for Average and High Consumers

Market	Vegetable	Percentage Lambda-Cyhalothrin	Percentage Cypermethrin
Soko-Mjinga	Kales	15.79%	84.21%
Soko-Mjinga	Tomatoes	25.75%	74.25%
Soko-Mjinga	Spinach	12.53%	87.47%
Ponda-Mali	Kales	25.77%	74.23%
Ponda-Mali	Tomatoes	13.45%	86.55%
Ponda-Mali	Spinach	8.78%	91.22%
Main Municipal Market	Kales	47.92%	52.08%
Main Municipal Market	Tomatoes	22.88%	77.12%
Main Municipal Market	Spinach	16.07%	83.93%
Average		**20.99%**	**79.00617%**

The results indicate that Cypermethrin contributes an average of approximately 79 percent to the hazard index compare to lambda-cyhalothrin that contributes an average of approximately 20 percent for both average and high consumers. The results suggest that low residue concentration of Cypermethrin in vegetables could have enormous impact on human health hence more attention should be given by the relevant authorities to the use and application of the pesticide

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

1. Sources of vegetables that supply vegetables to the three selected markets in Nakuru town within Nakuru County were; Njoro, Bahati, Subukia, Rongai, Molo, Nakuru town west and Nakuru town east sub-counties.

2. The mean consumption rates for Kales, Spinach and tomatoes were 0.116±0.111kg/person/day, 0.078±0.086kg/person/day and 0.107±0.052kg/person/day respectively.

3. The most common handling practices identified by respondents were;

    i. Buying vegetables that are already washed and chopped and cook them directly without washing them again.

    ii. Buying vegetables that are already washed and chopped and wash them again before cooking.

    iii. Buying whole vegetables that are not chopped, wash them and chop before cooking.

    iv. Buying whole vegetables that are not chopped, wash, chop and eat them raw as salad.

4. According to the results gotten from the study on handling practices and concentration of the two pesticides, consumers of spinach were at a high risk of exposure to the pesticides' residues.

5. The two most commonly used pesticides were Cypermethrin (21.21%) and Lambda-cyhalothrin (18.18%). The pesticides are both Pyrethroids and they are classified as moderately hazardous by WHO. They are used in kales and tomatoes.

6. The Estimated daily intake of Cypermethrin for average and high consumers was within the recommended limit of 0-0.02mg/kg bw except for high consumers of Spinach from main municipal market. The estimated daily intake of lambda-cyhalothrin for average and high consumers were within the recommended limit of 0-0.02mg/kg bw.

7. Hazard quotient of Cypermethrin for average and high consumers were below the recommended value of ≤1 except for high consumers of Spinach from main municipal market who exceeded the value hence significant negative health effects. Hazard quotient of Lambda-cyhalothrin for average and high consumers was below the recommended value of ≤1. Hazard Index for average and high consumers were below the recommended value of ≤1 except for high consumers of Spinach from main municipal market who exceeded the value hence significant negative health effects associated with the two pesticides. Cypermethrin contributed the most percentage to the hazard index.

8. The vegetables can be ingested in the quantity recommended by WHO apart from spinach which has a high hazard index. Therefore safe quantities for spinach is $0.07_{kg/person/day}$

5.2. Recommendations

1. Consumers should be encouraged to eat more vegetables as their consumption rate was below the recommended quantity by WHO.

2. Consumers should handle vegetables as required such as washing them with clean running water and other recommended practices to reduce exposure to pesticide residues.

3. The concerned authorities such as Ministry of agriculture livestock and fisheries, KEPHIS and KEBS should set and monitor regularly maximum residue levels of pesticides in locally consumed commodities to protect the consumers. The relevant authorities should pay attention to use of Cypermethrin since smaller amounts of the pesticide's residue results to great significance to human health.

4. Consumers should be encouraged to diversify their vegetable diets in order to prevent excess exposure to certain pesticides that are commonly used by farmers on specific vegetables.

5.3. Areas of further research

1. In this study, only lambda-cyhalothrin and cypermethrin were studied, hence more pesticides used in the vegetables should be studied.

2. The study did not assess the effect of various handling practices on pesticide residues, hence the practices should be assessed to determine their effect on residues.
3. Total diet studies that combines dairy products, fruits, other foods and beverages should be done in order to ascertain exposure to pesticide residues through all sources of dietary intake.
4. The study did not cover variability of pesticide use and residues in various season, hence this should also be studied further.

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APPENDIX I: STANDARD FOOD FREQUENCY QUESTIONNAIRE

My name is Teresa Mogoi Kenyanya. I am a student at Egerton University taking a Masters Degree in Environmental and Occupational Health. This questionnaire is for the purpose of collecting data and information to aid in a research that I am conducting. The information you provide will be confidential. Your assistance will be highly appreciated.

Registration number

Subject Code			**Sampling point**

Date: ………………………………………………………..

Section A: Background Information

Name of Respondent: ………………………………………………………………..

Respondent's gender: Male ☐ Female ☐

Respondent's age: ………

Level of education: ☐ None ☐ Primary ☐ Secondary ☐ Tertiary

Estimated income (KSh.) ☐

1=0-20,000, 2=20000-80000, 3=>80,000

Number of family members ☐

Section B: Most consumed vegetables

Which of the vegetables do you consume mostly?

Kales(sukuma wiki) ☐

Tomatoes ☐

Lettuce ☐

Cabbage ☐

Spinach ☐

Other (specify)

Section C: Vegetable consumption frequency and amount

Vegetable 1	***Consumption frequency and amount***	
	Frequency of consumption per week	
	Tomato weight in gms (frequently consumed)	
	Price	
	How many times do u serve the quantity bought?	
Vegetable2	***Consumption frequency and amount***	
	Frequency of consumption per week	
	Weight in grams (frequently consumed)	
	Price	
	How many times do u serve the quantity bought?	
Vegetable3	***Consumption frequency and amount***	
	Frequency of consumption per week	
	Weight in gms (frequently consumed)	
	Price	
	How many times do u serve the quantity bought?	

Thank You for Your Time and Cooperation.

APPENDIX II: QUESTIONAIRE FOR KEY INFORMANTS

My name is Teresa Mogoi. I am a student at Egerton University taking a Masters Degree in Environmental and Occupational Health. This questionnaire is for the purpose of collecting data and information to aid in a research that I am conducting. The information you provide will be confidential. Your assistance will be highly appreciated.

Registration

Subject Code			**Sampling point**

Date: ……………………………………

Section A: Personal Information

Name (optional): ☐ Subject Code ☐

Gender: Male ☐ Female ☐

Occupation: ………………………………………………………………..

Section B

What are the sources and pesticides used for these three most consumed exotic vegetables?

Vegetables	**Sources**
Kales (Sukuma wiki)	
Spinach	
Tomatoes	

APPENDIX III: QUESTIONNAIRE FOR FARMERS

My name is Teresa Mogoi. I am a student at Egerton University taking a Masters Degree in Environmental and Occupational Health. This questionnaire is for the purpose of collecting data and information to aid in a research that I am conducting. The information you provide will be confidential. Your assistance will be highly appreciated.

Registration number

Subject Code			**Sampling point**

Date

Section A: Background Information

Respondent's gender: ☐ Male ☐ Female

Respondent's age:

Level of education …………

i=Nursery, ii=lower-primary, iii=upper-primary, iii=secondary, iv=tertiary

Section B: Pesticides used

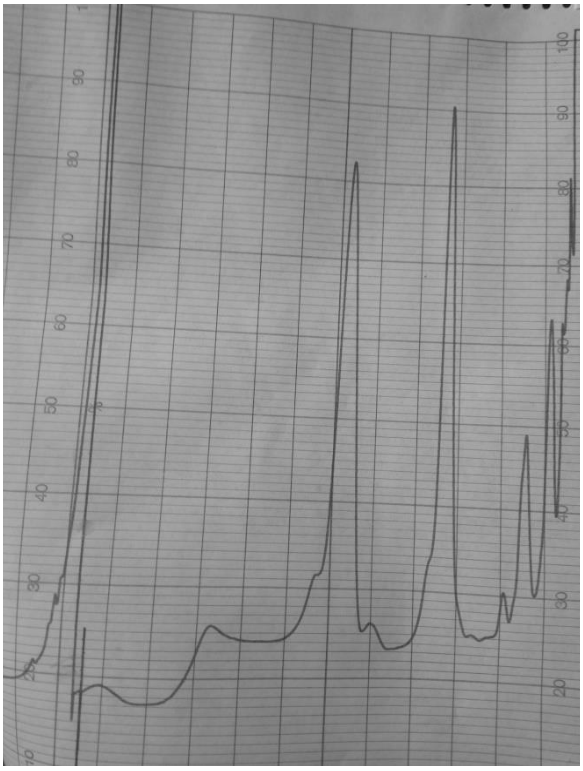
Vegetable	Pesticide used	Pre-Harvest Interval Period (Days)
Kales (Sukuma wiki)		
Spinach		

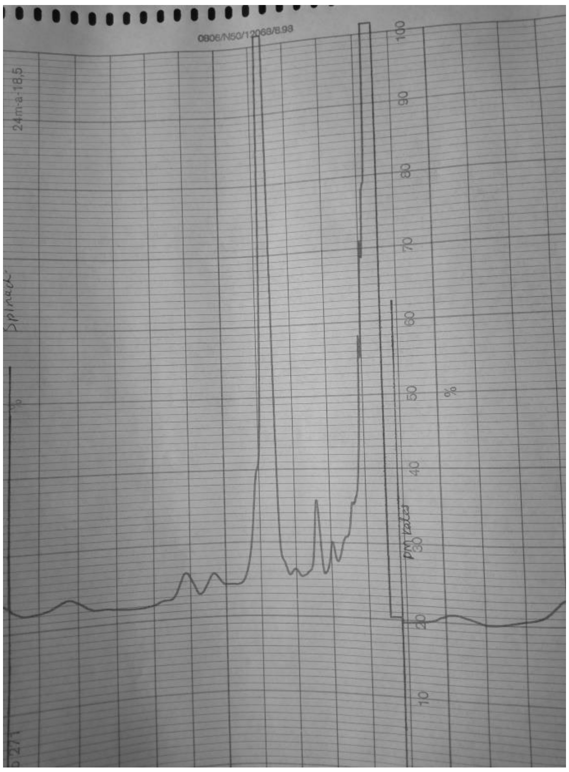
Tomatoes		

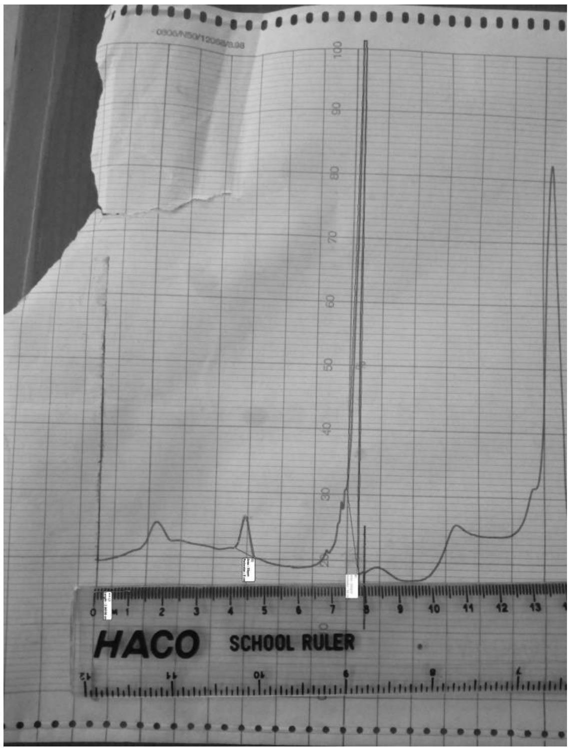
6. How often do you apply pesticide(s) to the vegetables after you harvest?
   ____

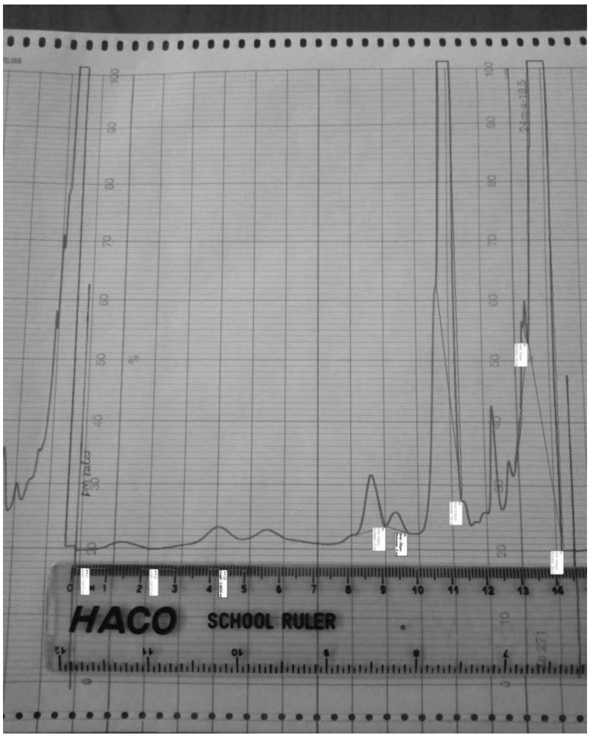
7. Do you read the instruction after buying pesticide and before spraying? Yes ☐ No ☐

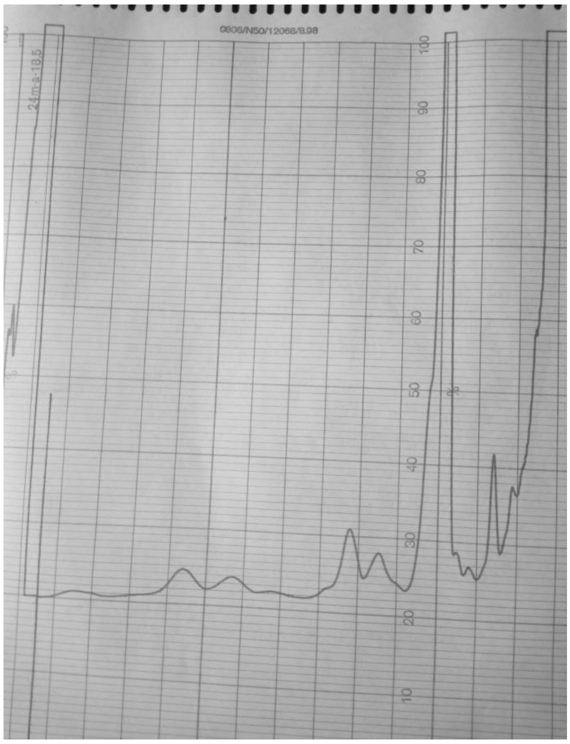
APPENDIX IV: CHROMATOGRAMS

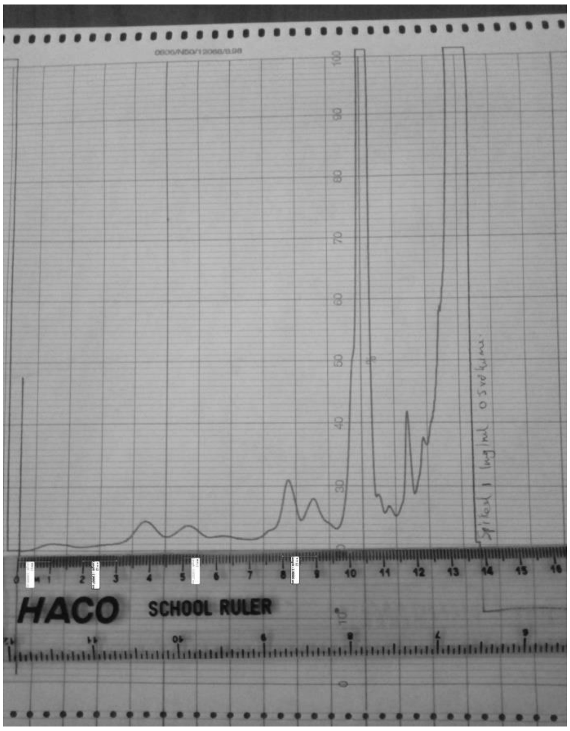


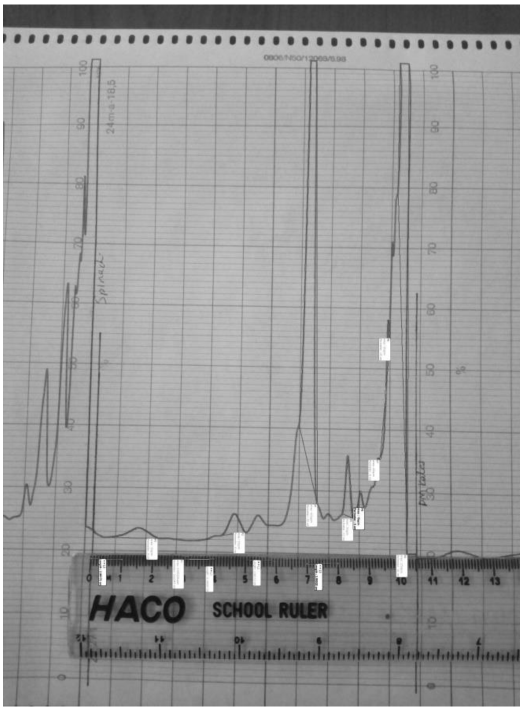












APPENDIX V: COUNTY PERMIT

P.O. BOX 15399-20100,
NAKURU.
+254726377219
tkmogoi@gmail.com.

TO:
COUNTY PUBLIC HEALTH OFFICER,
NAKURU COUNTY,
P.O. BOX 2870-20100
NAKURU.

Received & approved. Kindly submit a copy of your research to this office after completion

COUNTY PUBLIC HEALTH OFFICER
NAKURU

14/06/16

Dear Sir,

RE: REQUEST FOR PERMISSION TO DO DATA COLLECTION.

I would like to request for permission to conduct data collection for my postgraduate research.

I am a student from Egerton University taking a Masters of Science Degree in Environmental and Occupational Health in the Department of Environmental Science. My Research topic is on "Assessing Human Exposure to Pesticide Residues in Exotic Vegetables sold in selected markets in Nakuru Town, Kenya".

Your assistance will be highly appreciated. Thank you in advance.

Yours Sincerely

Teresa Mogoi Kenyanya.