

**DETERMINATION OF NEONICOTINOID RESIDUES IN HIVE PRODUCTS
FROM KIAMBU AND NAIROBI COUNTIES, KENYA**

BY

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**A Thesis Submitted in Partial Fulfillment for the Award of the Degree of Master
of Environmental Science in the School of Environmental Studies of Kenyatta**

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DECLARATION

Student's Declaration

This thesis is my original work, and has not been presented for a degree or any other award in any other University

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DEDICATION

I dedicate the work to all apiculture practitioners, my wife Mary, sons Mark and Emmanuel and late daughter Blessings.

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LIST OF ABBREVIATIONS AND ACRONYMS

BMPs	:	Best Management Practices
CCD	:	Colony Collapse Disorder
EMCA	:	Environmental Management and Coordination Act
ESI	:	Electrospray ion
FAO	:	Food and Agricultural Organization of the United Nations
GC	:	Gas Chromatography
GIS	:	Geographical Information System
GoK	:	Government of Kenya
ICIPE	:	International Centre for Insect Physiology and Ecology
KEPHIS	:	Kenya Plant Health Inspectorate Services
LC50	:	Concentrations that kills 50% of individuals
LC-MS	:	Liquid Chromatography-Mass Spectrometry
LD50	:	Dose that Kills 50% of individuals
MEA	:	Millennium Ecosystem Assessment
MRL	:	Maximum Residue Limits
NEMA	:	National Environmental Management Authority

OCLLE	:	On Column Liquid-Liquid Extraction
PCPB	:	Pest Control and Products Board
Ppb	:	Parts per billion
UHPLC	:	Ultrahigh Pressure Liquid Chromatography
UNCBD	:	United Nations Convention on Biological Diversity
UK	:	United Kingdom
EU	:	European Union
QuEChERS	:	Quick Easy Cheap Effective Rugged Safe
LOD	:	Limit of Detection
LOQ	:	Limit of Quantification
SD	:	Standard Deviation

ABSTRACT

Information on pesticide residue occurrence in hive products is scanty or lacking yet it is important so as to safeguard human health from effects of pesticides. The aim of the study was to identify and quantify neonicotinoid residues in hive products. The study was undertaken for 6 months between (March-August, 2015) at Kiambu and Nairobi Counties. The objectives of the study were to find out the pesticides used in the cultivation of crops, the frequency of use and the concentration of neonicotinoids in honey and bee bread (pollen). The methodology used was a structured questionnaire which was used to find out the pesticides used and frequency of their application. A modified Quick Easy Cheap Rugged Safe (QuEChERS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) were used to determine the presence and concentration of neonicotinoids. The Chi-square was used to test frequency of pesticide application on cultivated crops around the apiaries and T-test was used to perform a comparison between concentration of residues detected in honey, pollen and in different landscape structures as well as making comparisons with European Union Maximum Residue Limits (EU-MRL). The study results indicated commonly used pesticides are carbamates (32.4%), pyrethroids (14.6%), neonicotinoids (14.4%), herbicides (15.7%), fungicides (1.4%), acaricides (5.6%) and organophosphates (14.5%). Further, 26.4% of respondents used carbaryl carbamates, 13.2% use Karate, 12% thiamethoxam and 7.4% dichlorvos. Regarding frequency of application, 86% of respondents used the pesticides once every week, 12.5% fortnightly and 1.4% when available. Chi-square test showed no significant difference in the application frequency ($p > 0.05$). Honey was contaminated with acetamiprid with the mean levels of $0.41 \mu\text{g}/\text{kg}$. Thiamethoxam concentration in honey ranged from undetectable to $47.8 \mu\text{g}/\text{kg}$ in Thika IPM with mean of $19.81 \mu\text{g}/\text{kg}$ and standard deviation 24.77. Concentration of acetamiprid compound ranged from 0.1 ppb in Lari, Gatundu, Ruiru, Thika, Kikuyu, Karura and Ngong forests to 0.5 ppb in Thika IPM. The mean levels of neonicotinoid concentration in honey were found to be statistically significant when compared with EU maximum residue limits (50 ppb) established for food products ($p < 0.05$). The results generally showed low levels of neonicotinoid insecticides in bee food across Kiambu and Nairobi County. There was significant differences in neonicotinoid concentrations between cultivated and forested landscapes ($p = 0.009$) and also when compared with EU-MRL in food substances ($p = 0.001$). On average, there were higher concentrations of neonicotinoids in hive products from apiaries in cultivated areas compared to forested areas. Pesticides were detected in honey at remarkably low levels, ranging from 0.32 - 0.50 parts per billion (ppb), except for thiamethoxam, which measured 47.80 ppb in pollen. Based on the study results, honey from the studied areas is safe for human consumption and the honey bees are not exposed to harmful levels of neonicotinoids. However, pesticides persist in the environment and their levels should be monitored regularly. Beekeepers in the study areas are therefore advised to use the agro-ecological approach in applying the pesticides.

CHAPTER ONE: INTRODUCTION

1.1 Background to the Study

Pesticides are essential to farmers because they lessen crop losses due to insects, weeds, plant diseases, rodents among other pests. They also save lives through control of vectors and increase quality and quantity of agricultural yield. Pests in crop agriculture reduce crop production, quality and consequently contribute to widespread poverty especially in third world countries (Nderitu *et al.*, 2007). However, pesticides are toxic and can influence human health and pollute the environment.

Bees (*Apis mellifera scutellata*) are a key component of global biodiversity, providing important ecosystem services to crops and wild plants (Potts *et al.*, 2010; Biesmeijer *et al.*, 2006). They enhance pollination activities in agriculture (Carreck *et al.*, 2010). Pollinators contribute to food security and to the global economy (Aizen and Harder, 2009). Pollinators are also bio-indicators and are used to check environmental stress brought about by invasive species, diseases, vermin, predators as well as pesticides (Kasina *et al.*, 2010).

Internationally, pollination services amount to \$212 billion, equivalent to around 9.5% of the total value of global agriculture production for human use in 2005 (Gallai *et al.*, 2009). In East Africa, honey bees provide important pollination services, nutrition, and income for small- scale farmers and rural communities (Muli *et al.*, 2014). In Western Kenya, pollinators provide USD \$3.2 million in ecosystems services to beans, cowpeas, butternuts, sunflower, peas, tomatoes and passion fruit, (Kasina *et al.*, 2009). According to UNCTAD/WTO/ITC, (2005), Kenya is a net

importer of honey (over 10 metric tons in 2004, and thus honey production could be improved as a possible source of income for many rural families.

In Africa, pesticide use is generally lower when compared to other areas; this means honey bees are less frequently exposed to pesticides per food visit. However, South Africa is the highest food producing country on the continent and is also the largest consumer of pesticides in sub-Saharan Africa (PAN UK, 2007; World Bank, 2013). The use of the pesticides poses a serious issue to the country to develop satisfactory techniques which can combine optimal agricultural productivity and environmental safety (Musa *et al.*, 2011).

Honey and hive products (bees wax, venom, pollen, propolis) have the image of being natural, healthy and clean. During the last years, following the general trend of using what nature is directly offering, hive products are increasingly becoming important as essential natural resource in promoting good health, food and new therapy absolutely free from side effects of chemical medicines (Carmen *et al.*, 2001). However, nowadays hive products are produced in an environment that is highly polluted (Al-Waili *et al.*, 2012). Heavy metals, phosphorus containing pesticides and medicinal substances of veterinary value are considered among the important potential polluting agents (Bakan, 2002). Contamination can get to nectar, honey dew and pollen by air, water, plants and soil and transported into bee colonies by bees (Bogdanov, 2005).

Pesticides are well-known to cause environmental pollution and kill non-target organisms such as pollinators (Nderitu *et al.*, 2007). Pesticides also harm human health when their residues are consumed in food products. Pesticide residues in honey occur when bees searching for food, stopover crops that have been treated with pesticides and or when beekeepers use chemicals to treat or prevent diseases.

The significant public health hazards, range from short term effects such as headaches to chronic impacts like cancer and endocrine disorder (Berrada *et al.*, 2010). The use of poisonous pesticides, poor pesticides handling practices, inadequate regulation of these chemicals in third world countries by farmers has led to extra-ordinary public and environmental pollution (Gitonga *et al.*, 2010; Ntow, 2008; Waichman *et al.*, 2007).

Pollinator health is getting increased interest as both managed and local pollinator population reduces globally. In parallel, recent reports shows that over five to seven years, there has been momentous decline in the number of colonized hives leading to a decline in the size of migratory swarms and honey yield in Kenya (Mullet *et al.*, 2014).

This declining trend can be attributed to viral pathogens, parasitic mites and pesticides (Sammataro *et al.*, 2000). Neonicotinoid pesticides contact to honey bees continues to receive major attention because of their high efficacy, selectivity and plant systemicity (Elbert *et al.*, 2008). Neonicotinoids class of insecticides has become significant for use in agriculture and house landscapes (Jeschke and Nauen, 2011). They are relatively safe for people, animals and the surroundings (Tomizawa, 2004; Mohamed, 2009) because of their effectiveness and relative safety. They have properties of being deficient of cross-resistance to carbamates, organophosphates, or pyrethroids, against which many vermin have developed confrontation over the years (Jeschke, 2008). However, scientific research has provided evidence for and against possible connection to loss of pollinators (Blacquiere *et al.*, 2012). This has motivated environmentalists, governments and beekeepers to engage in an endless debate about whether or not a ban on pesticides would save the bees. When neonicotinoids were first introduced, beekeepers described different signs ranging and complexities

ranging from disoriented bees, bees swarming in small numbers on the ground, abnormal foraging behaviour, the occurrence of enormous bee losses in time of year, increased sensitivity to diseases and colony collapse (Whitehorn *et al.*, 2012).

The potential of pesticides to cause both short term and long term undesirable effects to the surroundings as well as public health has become an international concern. Several researchers have observed different residues of pesticides in honey and bee bread at varying concentrations (Blasco *et al.*, 2003, Choudhary and Sharma, 2008). The presence of the cumulative effects of pesticide residues in honey cannot only have bad effects on bees and people but they can also lower the quality of honey and diminish its beneficial qualities (Kujawski *et al.*, 2008). This implies that there is need to monitor the presence of neonicotinoid residues in honey to assess the potential health risk and to ensure that honey quality whether as food for humans or bees is kept intact.

Pesticides bring a guarantee of higher yields and freedom from diseases spread by vermin. However, pesticides are toxic and their residues do harm people and environment when concentrations are higher than the recommended residue limits (MRLs). If pesticides are left to contaminate hive products such as honey, nutrient rich food commodity for humans and bees, they result to diseases and death of pollinators. Therefore, pesticide residues have become a high concern problem in the field of food safety.

The presence of pesticide residues and other environmental pollutants in honey can have undesirable effects on bees and humans diminish the quality of honey and devalue its properties (Bogdanov *et al.*, 2008). So far several researchers have reported various residues in hive products at varying concentrations (Irani 2009;

Depinho *et al.*, 2010; Garcia- chao *et al.*, 2010; Blasco *et al.*, 2011; Weist *et al.*, 2011, Fontana *et al.*, 2010), confirming the need to constantly monitor pesticide residue in hive products to assess any likely health risks and ensure that its quality is not affected. To date only a few studies have been conducted to monitor pesticide residues in hive products produced from Africa (Eissa *et al.*, 2014). The pesticides have a biomagnifying property and if not monitored the recommended maximum residue limits may be exceeded and thus affect people and environment.

Therefore, this study was designed to fill the gap by identifying and quantifying neonicotinoid residues in honey and pollen (bee bread) in apiaries found in crop cultivated areas and forested areas in Kiambu and Nairobi County.

1.2 Statement of the Problem and Justification of the Study

Data on the status of the concentration of pesticide residues (neonicotinoids) on bee hive products (honey and bee bread (pollen)) is scanty or totally absent. Owing to their frequent use on cultivated crops and with new developed pesticides with new formulations that are potentially harmful to human health, it is important to monitor their concentrations in food products.

Beekeeping is practiced on small-scale basis as a source of income and source of food (proteins, vitamins, essential oils, antioxidants and sterols). Hive products (honey, propolis, pollen, royal jelly and bee venom have pharmacological), scientific technological, poetic and aesthetic culinary and cultural values in the study area (National Beekeeping Station, 2007). Most apiaries are located within the farms where crops such as coffee and flowers are heavily dependent on pesticides. The beekeepers depend on feral honeybees and colonies which emanate from swarms of

migratory bees and not reared queens, meaning many bees collect their pollen from plants sprayed with the pesticides. It is therefore important to monitor the amount of pesticides in the hive products.

The pesticides have a biomagnifying property and if not monitored the recommended maximum residue limits may be exceeded and thus affect human and honey bees' health. The study site supports a large horticultural industry both for export and domestic markets and this has consequently resulted to increased use of pesticides. Kiambu County was also chosen because it had adequate representation of small scale farmers (beekeepers), majority of whom were using pesticides. Though a lot of work has been done on pesticides in study sites, none of the studies have been done on determining neonicotinoid residues in hive products.

The study therefore aimed to identify and quantify neonicotinoid residues in honey and pollen (bee bread) in apiaries found in crop cultivated areas and forested areas in Kiambu and Nairobi County and suggests remedial measures to the problem.

1.3 Research Questions

To achieve the set out objectives, the study sought to answer the following research questions:

1. What are the types of pesticides applied and their frequency of application on cultivated crops around apiaries in forested (Ngong forest, Karura forest) and in cultivated (Juja, Thika, Thika IPM, Kikuyu, Ruiru, Gatundu and Lari) areas/landscapes?
2. Which are the neonicotinoids residues present in pollen (bee bread) and honey from apiaries located in forested and cultivated landscapes?

3. What is the level of contamination of hive products by neonicotinoid residues in forested and cultivated landscapes?

1.4 Research Hypotheses

The study was guided by the following hypotheses:

1. The types of pesticides, frequency of application on cultivated crops around apiaries in forested and cultivated landscapes are significantly different.
2. The concentration of neonicotinoid residues in beebread and honey from cultivated landscape is significantly high than in forested landscapes.
3. There is a significant difference in neonicotinoid contamination of hive products from apiaries located in forested and cultivated landscapes.

1.5 Objectives of the Study

1.5.1 General objective

The general objective of the present study was to identify and quantify neonicotinoid residues in hive products in selected apiaries in Kiambu and Nairobi Counties.

1.5.2 Specific Objectives

1. To identify the types of pesticides applied and their application frequency on cultivated crops around apiaries in Kiambu and Nairobi County.
2. To determine levels of neonicotinoid residues in hive products from apiaries located in forested and cultivated landscapes in Kiambu and Nairobi County.
3. To compare the concentration levels of neonicotinoids in hive products from apiaries located in forested and cultivated landscapes.

1.6 Significance of the study

This study makes an important contribution to an improved understanding of pesticide concentration in food products. The study generates specific information on the concentration of neonicotinoids in hive products. The information will assist policy makers, Ministry of Agriculture, NEMA, KEPHIS and other government agencies (PCPB) to come up with strategies on the best use of pesticides to protect honey bee populations from unnecessary pesticide exposure and teach the farmers on safe use of pesticides.

The results generate information on the type of pesticides used and this will be used by regulatory authorities to ensure that banned or restricted pesticides are not used by farmers. Decision makers in the ministry of trade and on the international level will benefit from the availability of current information in monitoring the quality of honey marketed locally as well as ensure that consumers are not exposed to pesticide residues in hive products. Finally, the information gathered could be used by other scholars as literature sources.

1.7 The Scope, Limitations and Assumption of the Study

This study focused on selected beekeepers distributed in six sub-counties of Kiambu and one in Nairobi. The study was carried out over a period of six months which may not have captured long-term changes or effect of seasons. The study was undertaken on the assumption that apiaries sampled represented other apiaries within the area.

1.8 Conceptual Framework

The study was aimed at identifying and quantifying neonicotinoid residues in hive products in selected apiaries in Kiambu and Nairobi Counties. The dependent

variables for the study included identification of presence and/or absence (level) of neonicotinoids, types of pesticide use and concentration of neonicotinoid in hive products. The frequency of pesticide use and application, type of landscape; forested and cultivated were treated as independent variables influencing the identification and quantification of neonicotinoid residues in hive products. The intervening variables included agronomic activities near honey bee foraging distance and apiary site and meteorological conditions during the sampling period. The study was conceptualized as illustrated in Figure 1.1.

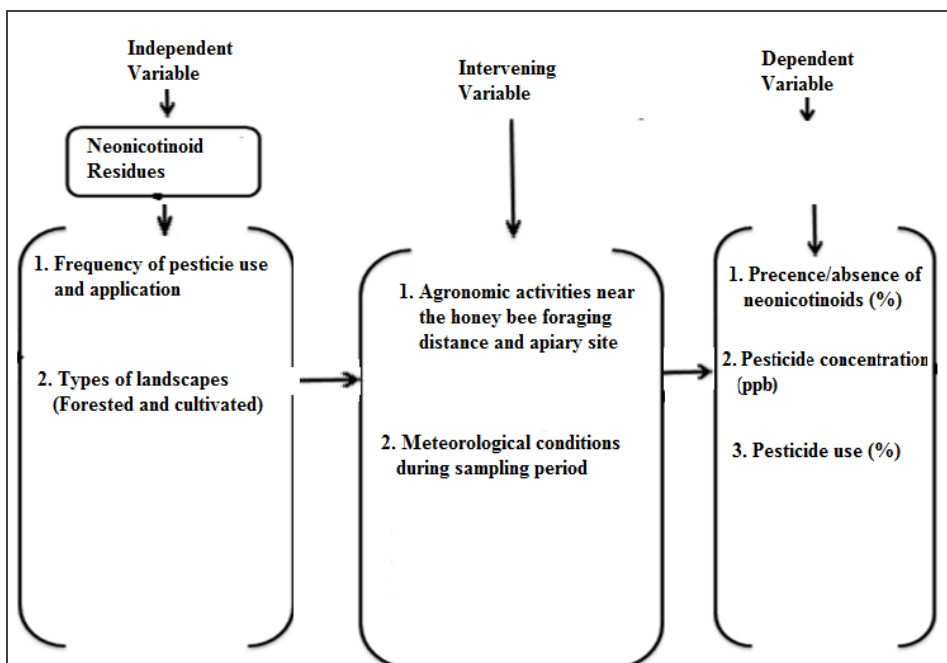


Figure 1.1: Conceptual Model of the Study

The presence and /or absence of neonicotinoids in hive products is influenced by a number of factors including period of application, agronomic practices near honey bee foraging distance and apiary sites as well as the sensitivity of the LC-MS/MS to detect and quantify the compounds in pesticide samples. Neonicotinoid residue concentrations is influenced by agronomic activities near the bee foraging distance,

year of use, the application frequency and landscape where the colonies are located and meteorological conditions during the sampling period, whether season of high or low pesticides application.

Pesticide use and application frequency is influenced by factors such as economic status of bee farmers, effectiveness of the pesticides and availability of the pesticide on the shelves being applied by the farmers. When hive products are contaminated to higher levels than those of international regulatory standards, management interventions policies need to be put in place to regulate on the use of neonicotinoids so as to ensure consumer and environmental safety, bees' product purity as well as improved bee health.

The research thus pursued three important objectives; to identify the types of pesticides applied and their application frequency on commonly cultivated crops around apiaries, to determine the presence of neonicotinoid residues as well as the possible differences in neonicotinoid residue concentrations. The finding of this study provides baseline data to guide formulation of policies to help influence appropriate conservation measures that can be implemented to protect the honey bees and humans from being poisoned by pesticides in Kenya.

1.9 Definition of Terms

Apiary, place where colonies of bees are kept for their products; honey, pollen, venom, propolis and bees wax.

Apis mellifera scutellata, the bee that is highly insistent and has a great propensity to reproduce and roam. It is found in plains and their high reproductive rate is attributed to enormous flowering, which occurs in the plains just before the rain season. They are best known of all bees because honey is obtained from them. They live in hives which can contain up to 50,000 bees.

Beekeeping is the art of keeping bees in order to attain honey, beeswax and other bee products for food, income and pharmacological products.

Bee bread, stored, fermented pollen assorted with nectar

Concentration, amount of pesticide or other chemicals in a quantity of liquid or solid (e.g. expressed as $\mu\text{g}/\text{kg}$).

Hive products, Pollen and honey, venom, bees wax, propolis

Neonicotinoids, these are insecticide compounds that act as nicotinic acetylcholine receptor argonist in insects, causing unrelenting excitation of these receptors and eventually death.

Systemic pesticides are chemical compounds transported through the plant to vascular systems making all tissues toxic to herbivorous insects looking for an easy meal

Residue, part of the fraction of pesticides that is left behind after the pesticide undergoes breakdown process.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter reviews cases of evolution of pollinator population decline as a problem of global concern, beekeeping in Kenya, importance of honey bees, effects of pesticides to honeybees and man, pesticide contamination of hive products; residues of pesticides in pollen and honey products, types of pesticides applied and application frequency. In addition, contaminations of hive products in cultivated and forested landscapes as well as contamination of bee products according to sampling period and environmental aspects of neonicotinoid residues are discussed. Moreover, appropriate conservation and policy measures for honey bees in Kenya including ecosystem approach to honey bee conservation are also discussed.

2.2 Evolution of pollinator population decline as a problem of global concern

Food production worldwide is dilapidated due to decline in bee pollination (Kasina *et al.*, 2010; vanEngelsdorp and Meixner, 2010). The reduction in pollination is due to multiple factors affecting pollinators among them are; parasites e.g. Varroa mites (Sumatra *et al.*, 2000), pathogens (Runckel *et al.*, 2011), pesticide exposure (Desneux *et al.*, 2007), poor nutrition (Brodschneider and Crailshein, 2010), reduced genetic diversity (Mattila and Seeley, 2007) and management practices (vanEngelsdorp *et al.*, 2012). It is unfortunate that information on pesticide residue occurrence in hive products is scanty, yet it is important to safeguard human health, environment and pollinators.

In the mid-1990s, scientists and farmers worldwide became concerned by a reduction in pollinators and their diversity. In order to sustain pollinators and their ecosystem

services, in depth understanding is required of the many goods and services provided by pollinators and the factors that affect their population decline and activity. It is vital to identify adaptive management practices that reduce negative impacts by people on pollinators, encourage the conservation and diversity of local pollinators, and conserve and restore habitats useful to maximize pollinator services in agricultural ecosystems. This situation provoked policy makers, at the fifth meeting of the Conference of the Parties to the United Nations Convention on Biological Diversity (United Nations Convention on Biological Diversity, 2000), to set up an International Initiative for the Conservation and Sustainable use of Pollinators.

2.3 Beekeeping in Kenya

Beekeeping in Kenya is becoming an important component of today's strategies for sustainable agriculture and integrated rural development programmes (Sammataro *et al.*, 2000). Beekeeping has a great potential for increasing income and sustaining development. It is of economic importance in that it is relatively cheap, self-reliant, it does not depend on importation of overseas equipment or inputs and beekeepers do not need large space in order to keep bees. Various products such as honey, bees wax, pollen, propolis, bee venom, royal jelly, queen bees and package bees and bee colonies can be sustainably obtained (Muli *et al.*, 2014). Beekeeping is therefore considered an agro-based enterprise that is able to develop strong linkages between biodiversity and sustainable source of revenue of the people (UNEP, 2006).

Moreover, it offers comparative advantage with positive environmental costs, bees are major pollinators and many ecosystems depend on them for their existence and for increasing their genetic diversity (Mattila and Seely, 2007; Fontaine, 2006). Beekeeping has potential for earning substantial foreign exchange and transforming

the living standard of farmers. With the introduction of modern beekeeping technologies; improved beehives and accessories, protective clothing and honey processing equipment including bee colony management, the industry has shown major progress in various aspects and is now a major component of the livestock segment (Mbae,1999).

In Kenya, honey and bees wax yield in 2007 was projected at 14,653 and 140 metric tonnes respectively valued at Kenyan shillings 4.43 billion per annum. The nation has a yearly estimated honey and beeswax yield potential of about 100,000 and 10,000 metric tonnes, respectively (Bio Trade Company, 2009). Despite this enormous potential the country is unable to meet its local market demand for honey and beeswax which is estimated at about 15,000 metric tonnes (Bio Trade Company, 2009).

The deficit was met through imports (49.932 metric tonnes of honey in 2008) while the country exported 7.579 metric tonnes of honey in the same period. Importation of such products requires the enforcement of hygienic regulations to avoid sub-standard products, introduction of bee infections and pests and free-for-all importation across the borders, which poses unjust competition to local producers. There also exists potential for the export market, but this has not been exploited due to low domestic characteristics of hive products (Bio Trade Company, 2009).

2.4 Importance of Honey Bees

Honey bees provide important ecosystem service, playing a key role in the safeguarding biodiversity, food and fibre yield through pollination (Kassinaet *al.*, 2010). Pollination comprises an integrated system of connections that link earth's

vegetation, wildlife and peoples wellbeing. This process is significant to fruit and seed production and is usually made available by insects looking for nectar and pollen. The health and safety of pollinators are essential to life, be it sustaining natural habitats or contributing to local and worldwide economies (Figure 2.1). Of all flowering plants on earth, 87.5% benefit from animal pollination globally, and eighty seven leading food crops depend on pollinators (Gallai *et al.*, 2009; Eilers *et al.*, 2011).

Honey bees can fly 4 km in all directions from their apiary and thus have access to an area of about 50 km². They are best small sampler that can be used in geochemical exploration. The honey bees have been used as bio-indicators for variety of environmental pollutants, including heavy metals (Holland and Turekian, 2003), low level radioactivity and pesticides (WHO, 1975; Nozal *et al.*, 2005).

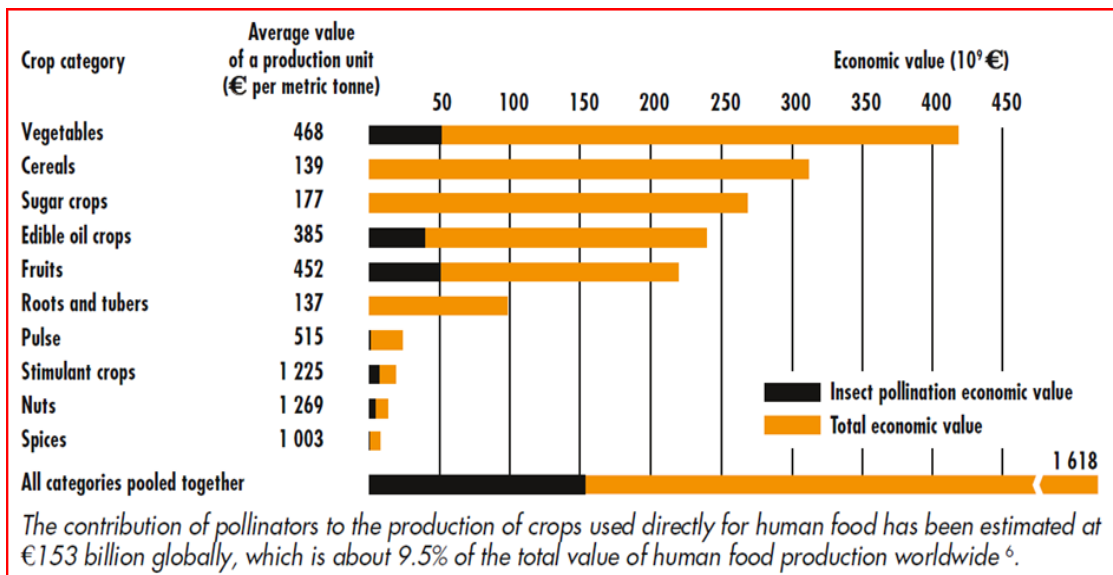


Figure 2.1: Economic impact of insect pollination on agricultural yield used directly for human food worldwide (Source: UNEP, 2006)

According to Gabriel and Tschamtket (2007), animal-mediated pollination improves the reproduction of natural plants on which other services or service-providing organisms depend. Some commercial plants, such as almonds or blueberries, do not produce fruit without the help of pollinators. A healthy-pollinated flower will be full of more seeds, with an enhanced ability to germinate, leading to bigger and better-shaped fruit. Improved pollination can also reduce the period between flowering and fruit set, minimizing the risk of exposing fruits to pests, disease and bad weather conditions.

2.5 Effects of pesticides on honey bees and man

Most pesticides and chemicals are not bio-degradable, and due to bio-accumulation, can enter into food chain and ultimately affect people, environment and animal health. Environmental exposure of pesticides to humans through ecosystems may occur during planting, agriculture practices, and consumption of food materials or air inhalation.

By transporting pollen from flower, honey bees play an critical role in the life-cycle of many of plant species (Breeze *et al.*, 2011). Honey bees have significant biological indicators of environmental contamination; they pick up chemical pollutants in the air or in flowers as they search for food (Beekmans and Ratnieks, 2000). For a long time, honey bee populations have been subjected to a global decline, which often is associated with colony collapse disorder (CCD) (Potts *et al.*, 2010; Van Engelsdorp, 2010). The main indication of colony collapse disorder is a radical loss of adult honey bees from the hives without any evidence of death. Frequently the queen bee, brood, food reserves and honey remain there in the empty hive.

Although diseases seem to be a causal factor to honey bee loss (Ratnieks and Carreck, 2010), it is alleged that honey bee population decline results from a combination of multiple factors. Among many factors, pesticides are highly supposed by researchers and beekeepers to have a great impact on honey bee death and colony weakening (vanEngelsdorp *et al.*, 2009). Moreover, many researchers have indicated that low levels of pesticides may stimulate unfavorable sub-lethal effects in honey bees (Henry *et al.*, 2012). However, the strategy for crop protection has been modified to improve food quality and safety, as a result there is rampant use of systemic insecticides via seed dressing, ensuing in lower amount of residues in pollen and nectar (Bonmatin *et al.*, 2003). The presence and the cumulative effect of pesticide residues in honey can not only have unpleasant health effects on bees and environment but they can reduce the quality of honey and its beneficial values.

2.6 Pesticides contamination of hive products

Pesticide residues in hive products occurs when bees forage on crops that have been treated with various pesticides and or when beekeepers use chemicals to take care of bee pests or prevent their attack such as *varroa mites* or disease (Eissa *et al.*, 2014). When apiaries are located in close proximity with horticultural environment and intensively cultivated farms, bees are likely to forage on polluted plants, carry pesticide polluted pollen and nectar into food chain through the hive matrix.

In Africa, pesticide use is generally lower when compared to other areas; this means honey bees are less frequently exposed to pesticides per food visit. However, South Africa is the highest food producing country on the continent and is the largest consumer of pesticides in sub-Saharan Africa (Alemayehu, 2001). The use of the pesticides poses a great test to the country to come up with satisfactory techniques

that can encompass optimal agricultural productivity and environmental protection (Musa *et al.*, 2011). For instance, neonicotinoid pesticides have been frequently associated with colony collapse disorder in Europe, Italy and USA among other countries (Creswell *et al.*, 2011; vanEngelsdorp *et al.*, 2010).

In East Africa particular data on the status of pesticide residues in hive products from major honey producing areas is scanty, yet it is important to protect human health and protect pollinators (Muli *et al.*, 2014). A recent study conducted by Muli *et al.*, (2014) in Kenya found a few pesticides in hive products at very low concentrations. However systematic introduction of pesticides into nectar and pollen may have direct consequences on honey bee health and ultimately result to pollution of hive products. Since the pesticides are highly persistent and bio- accumulates in food chain and become toxic to living organisms at higher trophic levels in the environment, they can pose health problems for honey bees and humans (Whitehorn *et al.*, 2012).

2.6.1 Residues of pesticides in pollen (Beebread)

Pollen constitutes the only source of proteins for the beehive (Kaskoniene *et al.*, 2010). The contamination of pollen can induce both contact and oral toxicity. The highest risk for honey bees is ingestion of imidacloprid as during the foraging on flowers, bees are enclosed with pollen (Stork *et al.*, 1999). Honey bees can encounter with pesticides when foraging or when the colonies is applied with pesticides to destroy mites as suggested by Carreck *et al.*, (2010). Foragers collect contaminated pollen and nectar in the field and on return they contaminate the hive. Nectar and pollen is mixed together with enzymes to make bee bread. In the hive bees disperse water from nectar to make honey (Tennekes, 2010). Honey bees heavily depend on nectar, pollen and other floral resources. When they forage on pesticide treated crops

their chances of exposure to residues of neonicotinoids or their metabolites increases (Henry *et al.*, 2012; Whitehorn *et al.*, 2012).

2.6.2 Residues of pesticides in honey

Honey is a sweet viscous liquid prepared by bees from nectar and honeydew collected from plant nectarines and processed before being stored by bees for food (Codex Alimentarius Standard, 1981; White, 1982). The composition and properties of honey are dependent on floral origins utilized by the bees and the climatic conditions of the area from which honey is harvested.

Studies show that the demand for residue-free honey, organic honey and other bee products continue to increase rapidly in the national, East Africa region and export market. In February 2002, the world honey market was heavily affected by a European Union (EU) outlaw on Chinese honey, following the identification and quantification of antibiotics in samples of Chinese honey. Since China was Europe's major supplier of honey, this resulted to a scarcity of honey meeting EU criteria, and honey prices went-up rapidly. The EU currently represents an outstanding market opportunity for small holder farmers/ beekeepers in African countries, with European and other buyers interested to buy more honey if it can meet EU criteria (Bradbear, 2009). Quality assurance in hive products is important in accessing both local, national and East African region and export markets. Currently, there is insufficient and inconsistent enforcement of existing standards. There is also poor coordination between public agencies charged with the task of quality assurance (Bio -Trade Company, 2009).

Honey production is seasonal depending on weather changes, there are two seasons. The first high season for honey production is between March and August and the second season is between September and January (International Trade Centre, 2004). Honey and honey products production is predominantly done by the small scale farmers in the arid and semi-arid areas of Kenya where other forms of agriculture cannot be sustained unless heavy investments on water systems is done. Apiculture farmers sell their products to local traders who consolidate beehive products on behalf of the processors. Alternatively, some farmers have formed organized groups which deal directly with the processors. Where this has happened, increased honey production has been observed (International Trade Centre, 2004).

Nowadays bee products are produced in an environment polluted by different sources of chemicals. Heavy metals, organophosphates, neonicotinoids, veterinary substances are considered to be among the important potential pollutants (Bakan, 2002). Contamination can reach the nectar, honeydew, pollen, plant exudates by air, water, plants and soil and then taken into the bee colonies by bees (Bogdanov, 2005).

Studies have shown that the level of contamination of bee products is highest in propolis followed by wax then pollen but least in honey (Bogdanov *et al.*, 2003). Studies in Switzerland and other countries have shown that contamination of honey products is generally low, thus showing negligible health hazard to consumers. However, they may have implications for honey bee health. Honey bees seem to have a filtering effect since honey is less contaminated. Pollen, wax and propolis, on the other hand, are said to have higher concentrations of pollutant (Bogdanov *et al.*, 2003).

The damage to the honey bee colonies by pesticide use is inclined not only by the toxicity and potency of the pesticides, frequency and methods used during application, time and season when application occurs, the prevailing metrological conditions, but also by the number of honey bees in the hive visiting flowers in the treated environment, the type of nectar or pollen collected, type of food flowers and even the influence of food consumed by honey bees for the weeks after crop have been treated by pesticides (Williams *et al.*, 2010).

A recent survey carried out in Nyeri County in Kenya, showed that farmers used a dose above the recommended one in their effort to reduce pest damage (Gitonga *et al.*, 2010). This is a reflection of the indiscriminate use of pesticides by farmers in Central Kenya. Most farmers are using Thunder with an active ingredient imidacloprid / betacyfluthrin) at a dose well above the recommended level to eliminate of leaf miners (Lekei, 2014)

2.7 Types of pesticides applied and their application frequency on cultivated crops around apiaries

Many pollinator dependent crops such as; beans, cowpeas, butternuts, sunflower, monkey-nuts, tomatoes, capsicum and passion are grown near apiaries for effective pollination services, (Kasina *et al.*, 2009). Apiculture is currently one of the most widespread agricultural activities carried out throughout the country and location of the apiaries is becoming quite a big problem. Many colonies are highly contaminated when located in apiary sites in high potential areas characterized by horticultural, floricultural farming, large scale coffee and tea plantations. These kinds of crops are heavily dependent on pesticides for pest control. According to Yadav (2010), without

pesticides or agricultural chemicals, crop yield could reduce by as much as a third and food prices would increase by as much as 75%.

Pesticides can be classified according to their active ingredients specifically; organochlorines, organophosphates, carbamates, pyrethroids, neonicotinoids, inorganic among others (Louis, 1994). Organochlorines are stable and slow degrading once applied and is soluble in fats and accumulates in the adipose tissues of receptor organisms (Marthur *et al.*, 2005). They are passed up the food chain where they bio accumulate in fatty tissues.

Organophosphates are more poisonous than organochlorines though they are easily hydrolysed but are highly toxic to invertebrates and insects such as bees (Chambers *et al.*, 2010). Carbamate are fat soluble and therefore easily engrossed through the skin and then move to the entire body system. Carbamates are extremely poisonous to bees and parasitic wasps (Liu *et al.*, 2012). The World Health Organization (WHO) classifies pesticides based on their toxicity; as extremely hazardous (class IA), highly hazardous (class IB), moderately hazardous (class II), slightly hazardous (class III) and unlikely to present acute hazard (class IV) (WHO, 2008).

Pesticides are among the priority pollutants to be monitored in a wide variety of matrices due to their amalgamation into foods, waters and soil, which may signify a potential health hazard to humans (Chuanjiang *et al.*, 2010). Insecticides are generally the mainly toxic pesticides to the environment, followed by fungicides and herbicides (Yadav, 2010). Exceptions exist for certain herbicides which are highly toxic, and are far more hazardous to the environment than insecticides (Yadav, 2010).

According to Nyamu (2008), there is an increasing use of chemicals and pesticides among Kenyan farmers. The government is paying attention on boosting crop yields yet there is lack of regulation and information to direct users on the use and application, health and environment impacts of the pesticides. In the rural areas, the main concern is the indiscriminate use of pesticides. Certain pesticides are used widely in small-scale agricultural activities and are so toxic that their use is either banned in other countries such as Canada. Kenya's importation and use of pesticides has more than tripled in the last ten years, but majority of farmers do not handle the hazardous pesticides safely (Nyamu, 2008).

The use of pesticides has definitely increased agricultural outputs, and improved prolonged existence and human well-being. Coupled with these successes are a number of side effects. Pesticide use is still indispensable in Kenya in the area of agricultural production and public health vector control. However, the toxicity of these compounds and their presence in the environment pose serious issues that necessitates the development of methods that will increase agricultural productivity and control vectors with minimal environmental contamination and negative effects to non-target organisms (Musa *et al.*, 2011). The use of the pesticides poses a great challenge to the nation to develop satisfactory techniques that involve combination of optimal agricultural productivity and environmental protection (Musa *et al.*, 2011).

2.8 Contamination of hive products in cultivated and forested landscapes

Honey bees are constantly exposed to pesticide particularly if their colonies are located in intensive agricultural areas. Most studies have compared the contamination in both urban and wild sites and results have revealed that apiaries in rural- cultivated landscape were more contaminated than apiaries in all other landscapes (Balayiannis 2008; Perugini *et al.*, 2011; Lambert *et al.*, 2012). However, part-per-billion residues (ppb) and occasionally parts per million (ppm) residues levels can be detected in hive products such as pollen, beebread, honey, when honey bees forage in intensive conventional cropping or urban environment. Honey or pollen contaminated at ppb residue levels with neonicotinoids which are known to destroy honey bee health (Decourtye *et al.*, 2004; Halm *et al.*, 2006; Desneux *et al.*, 2007).

2.9 Contamination of bee products

Previous studies have shown that the highest season of contamination match up to late April-early May and were associated with intensive agricultural use and application of pesticides and crop treatments and high foraging activity (Krupke *et al.*, 2012). Ghini *et al.*, 2004, who carried out samplings every month from April to October, 2000 showed that the higher contamination in spring of 2008 was not observed in 2009, most likely due to differences in meteorological environment. Other studies have demonstrated seasonal variation in beehive products contamination by polycyclic aromatic hydrocarbons (Lambert *et al.*, 2012) and heavy metals (Morgano *et al.*, 2010; Perugin *et al.*, 2011).

2.10 Neonicotinoids and Derivatives

Neonicotinoids are derived from wild plant compound nictines with insecticidal properties. Neonicotinoids attach more strongly to insect neuron receptors than mammal or vertebrate neuron receptors. These insecticides are selectively more pousous to insects than mammals. Six neonicotinoid compounds; dinetofuranclothianidin, imidacloprid , acetamiprid,Thiacloprid and thiamethoxam are approved for use as pesticides in United Kingdom (UK) and European Union (EU), while in Kenya imidacloprid , acetamiprid and thiamethoxam have been approved for use in controlling insect pests in coffee farms, french beans, maize, cotton, wheat, forestry nurseries, roses, tobacco and vegetables (PCPB,1998). Of these, imidacloprid and thiamethoxam have similar chemical makeup and are acute toxicity to bees whereas; acetamiprid and thiacloprid are much less acutely poisonous and structured differently.

Many insecticides are now regularly used to protect crops against plant eating insects. The most applied ones have been imidacloprid and organophosphorous pesticides (Pettis *et al.*, 2010). Imidacloprid is a systemic neonicotinoid insecticides used for coating sunflower seeds (Decourtye, 2004). The widespread use of imidacloprid as a systemic insecticide, and its likely movement to pollen and water, has raised concerns for the potential detrimental impact to pollinators (Bacandritso *et al.*, 2010).

Systemic neonicotinoid use has greatly increased recently for treating seeds of genetically-engineered crops (Halmet *et al.*, 2006) and they have a considerable impact on non-target species such as pollinators (Mullin *et al.*, 2010). Imidacloprid is the most used active ingredient of the neo-nicotinoid , and successfully protects the whole plant. It is vigorous against; rice hoppers or aphids, as well as other insects such as

thrips, whiteflies, termites, turf insects, and beetles. This compound is most usually used on roses, coffee, rice, maize, sunflowers, potatoes, vegetables, and fruits crops (Dai *et al.*, 2002; Beivier *et al.*, 2003).

Imidacloprid interferes with the insect's nervous system by blocking nicotinic neuronal pathway and interfering with transmission of stimuli. This pathway is extensive in insects than in warm-blooded animals, making the chemical more poisonous to insects than to mammals (Tomizawa *and Casida*, 2003). Imidacloprid protects roots and shoots after seed has germinated. The entire plant is also protected during its growth period because the systemic imidacloprid is carried by the sap into the many parts of the crop. However, the level of imidacloprid reduces as growth and development continues and very low levels are noticed during the flowering stage.

Studies have shown that concentration of 1-5ug/l of neonicotinoids in nectar appears to be the limit below which no effect is detected (Creswell *and VanEngelsdorp* 2012) and most residue determined in nectar and pollen of treated crops are normally at or below this limit (Blackquiere *et al.*, 2012). In addition, examination of bee foraging behaviour indicates that bees are likely not to feed on treated crops treated with pesticides(Thompson *et al.*,2013), thus lessening any effects of neonicotinoids.

2.11 Appropriate Conservation and Policy Measures for Honey Bees in Kenya

The Government of Kenya has developed policies to prevent habitat loss through the creation of the Environment Management and Coordination Act (GoK, 2015) which has also encouraged private conservation of wildlife, Forest Act (GoK, 2006) and the Wildlife Act (GoK, 2013). Recently the Government of Kenya developed a policy to

encourage all farmers to set aside at least 10% of their farm for trees and other life forms.

Many farmers are now developing structures to implement the policy (Holzschuh *et al.*, 2008; Hannon *et al.*, 2009). While habitat loss is a key threat, bees are able to survive by utilizing farm pockets suitable for nest establishment and presence of food resources all the year round. However the current agricultural practices used do not support the safety pockets (NEMA, 2011).

The National Environmental Management Authority (NEMA) is responsible for promoting sustainable environment management; whereas the Pest Control Products Board (PCPB) functions under pesticides control act (CAP346), which regulates the manufacture exportation, importation, distribution and the use of pest control products. According to Wandiga (2001) and Lenkei (2014) pesticides that have been banned or have restricted are still available in the Kenyan market where farmers can easily purchase and use them. The high use of a wide range of synthetic broad spectrum pesticides is common, this contributes to decline of bees, their foraging and nesting sites. Main tillage practice used by farmers is soil pulverization which has a harmful effect on soil nesting bees (Bommarco *et al.*, 2010).

Farmers still harbour a perception that bees are pests, they use pesticide to get rid of them (Kasina *et al.*, 2010). However they should be encouraged to scout for any signs of pests' not bees very early and control pests before they spread to other areas. This will reduce pesticides use and hence increase pollination since they will be conserved. Integrated pest management (IPM) is an environmentally friendly approach to pest management, daunting the use of pest control methods that have negative effects to the non-target species. Using synthetic pesticides, within IPM, should only be used at

lowest levels and sensibly as suggested by Williamson *et al.*, (2008). In Kenya, and most African agricultural systems, IPM is not given precedence, particularly through government policies. The use of the pesticides is a great issue of concern to the nation to develop satisfactory techniques that can bind optimal agricultural output and environmental protection (Musa *et al.*, 2011).

2.12 Ecosystem approach to honey bees conservation

Ecosystem approach is linked to the application of relevant scientific methodologies focused on levels of biological structure, which entail the essential structure, functions, processes and interactions among organisms and their environment. It recognizes that mankind, with their cultural diversity, are an integral part of various ecosystems. It is a strategy for the integrated management of land, water and living resources that promote conservation and sustainable use in an equitable way.

The application of the ecosystem approach in the conservation of honey bees will also help to reach a balance of the three objectives of the United Nations convention on Biological Diversity (United Nation Convention on Biological Diversity, 2000) which are; conservation; sustainable use; and the fair and equitable sharing of the benefits arising from the utilization of mobile/genetic resources. In addition the ecosystem approach has been recognized by the World Summit on Sustainable Development as an important instrument for enhancing sustainable development and poverty alleviation (United Nations Convention on Biological Diversity, 2000).

Farmers and gardeners can rely on alternative non-poisonous control methods such as natural enemies and eco-friendly practice to control pests and weeds, therefore reducing exposure to honey bees of insecticides, organophosphates,

neonicotinoids, herbicides and fungicides (Corbet, 1995). It is imperative that the impacts of pesticides on pollinators are considered when designing and choosing methods of pesticide use and application, specifically during the flowering period in apiary sites surrounded by pollinator-dependent crops.

Maximizing pollinator-friendly variety of plant species offers improved forage opportunities for pollinators (Carvell *et al.*, 2006; Kremen, 2007) and may also strengthen pollinator movement, colonization and persistence in restoration initiatives. However, due to threats on honey bees by pesticides, the pollination element is threatened. Systemic pesticides are widely used by farmers and bee keepers to control pests and their impact on bees remains unclear. Similarly, raising awareness to farmers remains a big challenge, especially the perception that bees are also pests. The effects of intensive use of agricultural pesticides on honey bees, if not adequately addressed, may continue to threaten global food security.

2.13 Knowledge Gaps in the Literature Reviewed

From the literature reviewed in this chapter, the research gaps are as follows; first, most developing countries, farmer's knowledge about pesticides and available alternative is limited and short term cost considerations still remains an important factor in poor farmers choice of pesticides. Secondly, banned pesticides or that have restricted use in the Kenyan market and are sold freely over the counter. Thirdly, in many veterinary shops without any proper monitoring or adherence to the stipulated restrictions on its sale and application. Fourth, bees colonies located in intensive agricultural areas are more contaminated than in forested landscapes, beekeepers have very little knowledge on contamination of apiaries located in horticultural farms. Fifth, there is no homogeneity on Maximum Residue Limits; different National

regulatory bodies have established their concentrations of pesticide residues permitted in honey and lastly, information on the levels of neonicotinoid pesticide residues in hive products in Africa is scanty or totally absent yet such knowledge is important to safeguard human health and conserve pollinators.

This study attempts to identify and quantify neonicotinoids residues in hive products under field study. Therefore, this study departs from previous studies by focusing on determining neonicotinoid residues in African honey bees' (*Apis mellifera scutellata*) hive products in selected apiaries located in forested and cultivated landscapes in Kiambu and Nairobi County, Kenya in detail.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction

This chapter describes the study area, climate, soil and relief, economic activities and population of the study area; procedures of collection of sample collection and analysis of neonicotinoid residues in honey and pollen.

3.1.1 Study Area /location

The study was carried out in Kiambu County located between latitude 1°20' S- 0°55'N and longitude 36°30'E-37°20'E approximately 1600m above sea level. The county consists of Juja, Kikuyu, Limuru, Githunguri, Kiambaa, Thika, Lari and Ruiru sub-counties (Figure 3.1). It is approximately 15km North of Nairobi, the capital city of Kenya. In Nairobi county samples were collected at Ngong and Karura forests which falls within latitude 1°17' S- 0°55'N and longitude 36°43'E approximately 1100m above sea level (Figure 3.1).

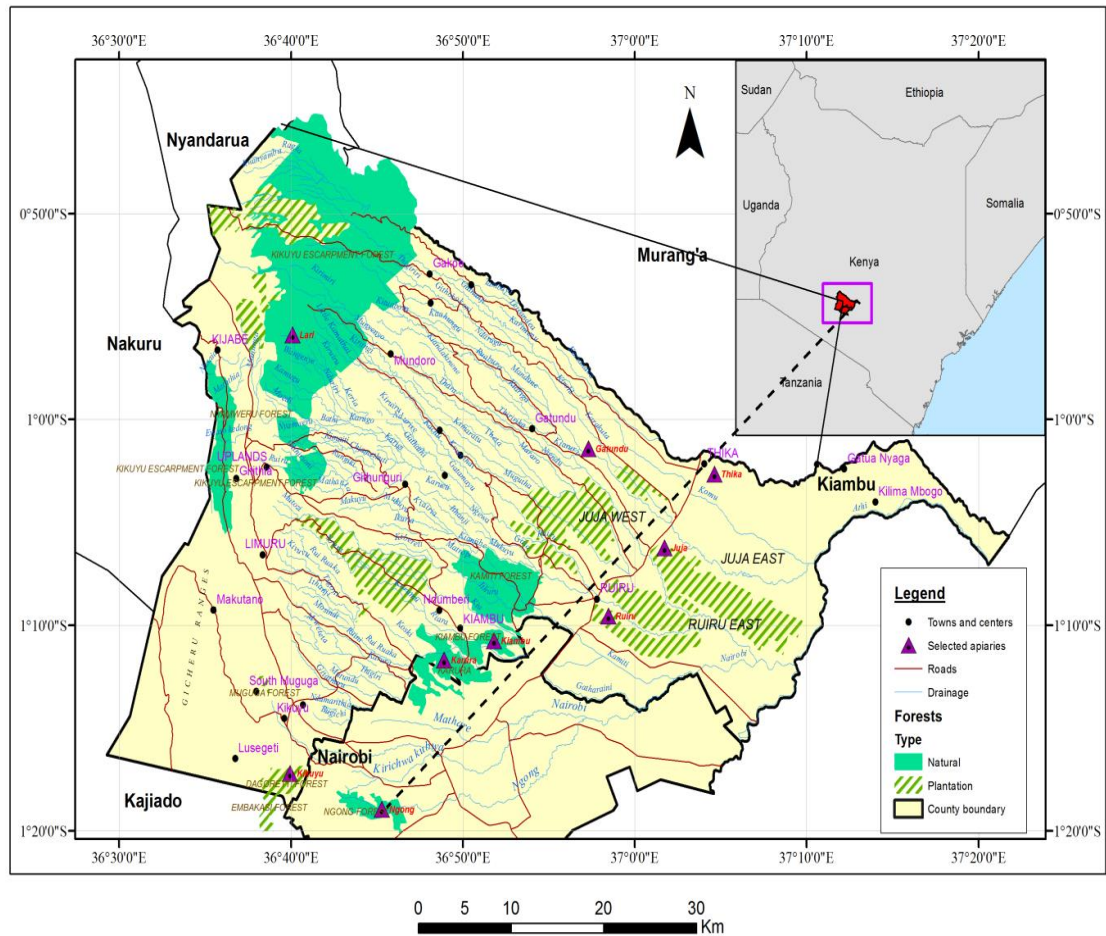


Figure 3.1: Map of Kenya showing the selected apiary sites in Kiambu and Nairobi Counties. (Source: Google map, 2016)

3.1.2 Climate, Relief and Soils

The farmlands of Kiambu and Nairobi Counties consist of rich agricultural soils with an average rainfall of 989 mm per annum. The counties have a bimodal type of rainfall pattern, with long rains occurring between April to May and short rains in October to November. The mean monthly temperatures range from 12.8°C to 24.6°C with an average daily temperature of 18.7°C. Higher areas have rich soils suitable for tea, coffee, and dairy; lower area is suitable for cereals and horticultural crop production (County Government of Kiambu, 2013).

3.1.3 Economic Activities

The main economic activity is small scale farming which provides a livelihood for 75% of the population (Muli et al., 2014). The sector comprise of horticulture and floriculture farming, large scale coffee and tea plantations, subsistence farming around the tea and coffee plantation farms (HCDA, 2010). Main crops grown are coffee and flowers (roses) which heavily depend on pesticides. Other crops grown include pineapples, maize, beans, pumpkins, tomatoes and vegetables. Beekeeping is estimated to be practiced by 400 farmers mainly on small-scale basis. Farmers with hives do not rely on rearing queens or colonies but on feral honeybee colonies emanating from swarms of migrating bees. (National Beekeeping Station, 2007).

3.1.4 Population

Kiambu County has a population of 1,623,282 that live in urban or peri-urban set up. Age distribution include; 0-14 years (34.5 %), 15-64 years (61.9 %) and above 65years (3.6%) (KNBS, 2010).

3.1.5 Vegetation

The vegetation in the study area is dominated by natural forests and cash crops. The study areas have various forests which include Ngong, Karura, Ololua forest and Nairobi arboretum. The dominant tree species include; *Olea europea*, *Croton megalocarpus*, *Warburgia ugandensis*, *Brachyleanahullensis*, *Uvaridendronanisatum*, bamboo trees and *Lantana camara* as invasive species. Ngong forest covers an area of 638 hectares, Karura forest 1063 hectares, Ololua forest 667 ha, and Nairobi arboretum 25 ha. The forest cover is receding due to urban sprawl, construction of roads and demand of food for the growing population (UNEP, 2006).

3.2 Selection of Apiaries

The actual study was preceded by a preliminary survey in the month of March, 2015. The purpose of the pilot study was to familiarize with the study area, establish contract with beekeepers, and test the questionnaire and instruments to be used in the actual study. The preliminary study found a total of fourteen apiaries. Two apiaries were located in forested landscapes of Ngong and Karura free from high levels of anthropogenic activities. Twelve apiaries were distributed in cultivated landscapes of Juja, Thika, Thika IPM, Gatundu, Kikuyu, Ruiru and Lari. Cultivated landscapes had large fields of crops (oil seed, grain crops, market gardening and horticultural crops). Consequently, out of the 12 apiaries, three apiaries in cultivated landscapes were found to be inaccessible as the home owners had warned of trespassing, two were also located very close to homesteads and sampling them was risky to family and livestock. The remaining seven apiaries therefore were purposively chosen for the study, two in forested area and seven in cultivated landscapes.

3.2.1 Sample Size and Sampling Procedure

Honey and pollen samples were purposively collected from nine apiaries. In each apiary, five colonies were identified, but three were randomly sampled. Honey and pollen samples were pooled separately into two samples of honey and pollen respectively and put in 50ml Falcon tube and transferred to the laboratory in a refrigerated cool box at 4⁰C and kept at -80⁰C while a waiting for analysis. A total of nine pooled honey samples and nine pollen samples were collected and analyzed during the study period (Figure 3.2).

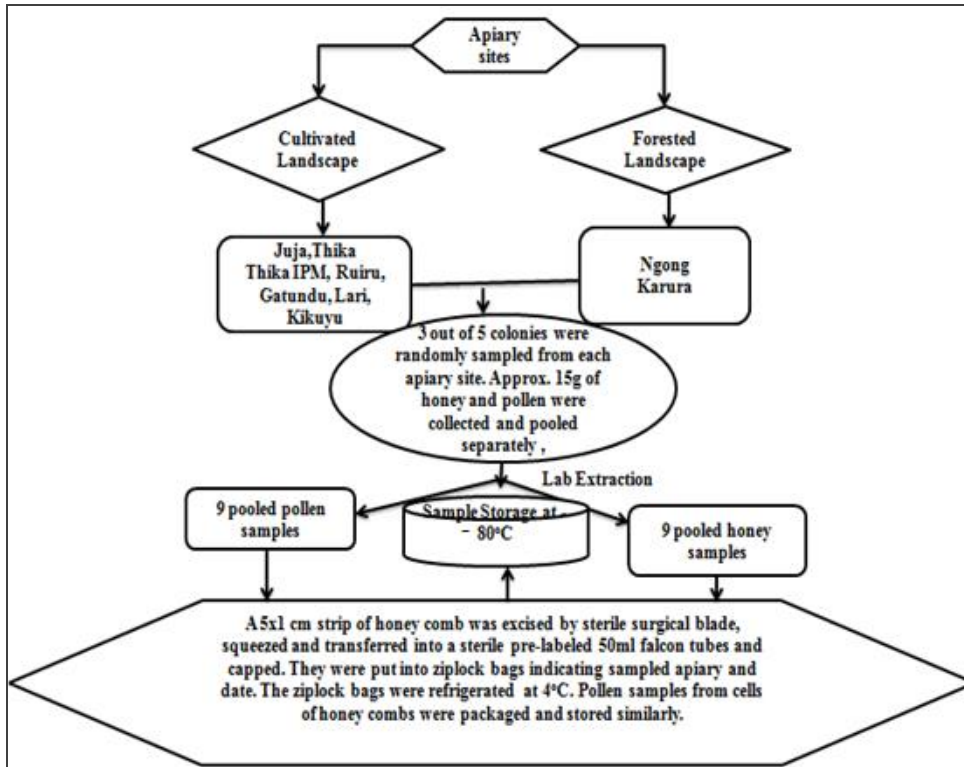


Figure 3.2: Schematic diagram showing sample size and sampling procedure

3.2.2 Honey Samples Collection and Storage

Prior to sampling, 5-10 puffs of smoke were blown into the hive to calm the bees, the hive was then opened and a frame with honey comb removed. A 5x1cm strip of honeycomb (approximately 15g) was excised using a sterile surgical blade. The honey was squeezed out and then transferred into a sterile labelled 50ml Falcon tube and capped and put into respective sealable Zip lock freezer bags which were labelled to indicate the sampled apiary and date of sampling. The zip lock bags were placed in a refrigerated cool box at 4°C and transported to the laboratory. At the laboratory, samples were stored in a standard freezer at -80 °C freezer ready for neonicotinoid pesticide residue analysis (Janet *et al.*, 2016).

3.2.3 Pollen Samples Collection and Storage

At the site, prior to sampling (Plate 3.1), 5-10 puffs of smoke were blown into the hive to calm the bees, the hive was then opened and a frame with honey comb removed. A 5x1cm strip of honeycomb (approximately 15g) was excised using a sterile surgical blade. The pollen (beebread) was collected from cells of honey combs. The pollen crystals were transferred into a sterile labelled 50ml Falcon tube and capped and put into respective sealable Ziploc freezer bags which were labelled to indicate the sampled apiary and date of sampling. The zip lock bags were placed in a refrigerated cool box at 4⁰C and transported to the laboratory. At the laboratory samples were stored in a standard freezer at -80 °C freezer ready for neonicotinoid pesticide residue analysis (Janet *et al.*, 2016).



Plate 3.1: (a) preparing to collect samples at Karura forest, (b) Identified colonies for Sample Collection at Thika Apiary

3.3 Data Collection Procedure

The study was structured in three stages. The first stage involved a field study that was aimed at identifying the type of pesticides applied and their application frequency on cultivated crops planted around apiaries. This was conducted through questionnaires administered randomly to 72 beekeepers (Appendix II). Simple random sampling was employed as it ensures that each member of the target population has an equal and independent chance of being included in the sample (Burton and Bartlett, 2009). This method is used when the population is uniform or has similar characteristics and the sampling frame is known (Walliman, 2005).

The sample size was 72 beekeepers, determined using the formulae $n = \frac{z^2 \cdot p \cdot q}{e^2}$ (desired sample size / $1 + n/p$ (population size)), (Mugenda, 2008) because the sample size was less than 10,000, in this case 400 beekeepers.

The second stage involved collecting samples of honey and pollen (bee bread) from selected apiaries for a period of five months between April, 2015 and August, 2015. This time frame coincided with agricultural activities including harvesting of honey and heavy pesticides application. It was also observed to be a period of high foraging activities by honey bees. In the third stage, the samples collected were pooled and transported to the laboratory at 4°C and immediately stored at -80°C away from direct sunlight and thereafter processed for analysis (Janet *et al.*, 2016).

3.4 Samples Extraction and Laboratory Analyses

Sample extraction and analyses was carried out in African Reference Laboratory for Bee health, International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. Prior analysis of honey and pollen samples, obtained from the local organic

farmers from Kenya was performed to ensure that it did not contain any of the studied pesticides. The samples were selected as a blank (reference samples) for spiking, preparing matrix matched calibration curves and for recovery purposes. Samples were prepared following the QuEChERS method (Anastassiades, 2013).

3.4.1 Extraction of Honey

Five grams of blank honey sample was weighed into 50ml falcon tube separately and spiked with 0.1ug/kg of neonicotinoids and prepared along with the samples for recovery evaluation as a control. A portion of 10ml of LC-MS/MS grade water was added and the mixture was shaken for one minute followed by 10ml acetonitrile and vortexed for one minute again. A mixture of salts (4g of magnesium sulphate, 1g sodium chloride, 1g trisodium citrate dehydrate and 0.5g of disodium hydrogen citrate sesquihydrate) was added to induce phase separation then vortexed for one minute and centrifuged at 4,200rounds per minute(rpm) for 5 minutes.(Figure 3.3). A 1ml aliquot of the final solution (supernatant) was filtered using 2µm filter membrane. 200µl of filtered honey was transferred into another vial and 200µl LC-MS/MS grade water was added to dilute the honey before the final solution was transferred to an auto sampler vial (1.5ml) for pesticide analysis using LC-MS/MS. (Anastassiades *et al.*, 2003).

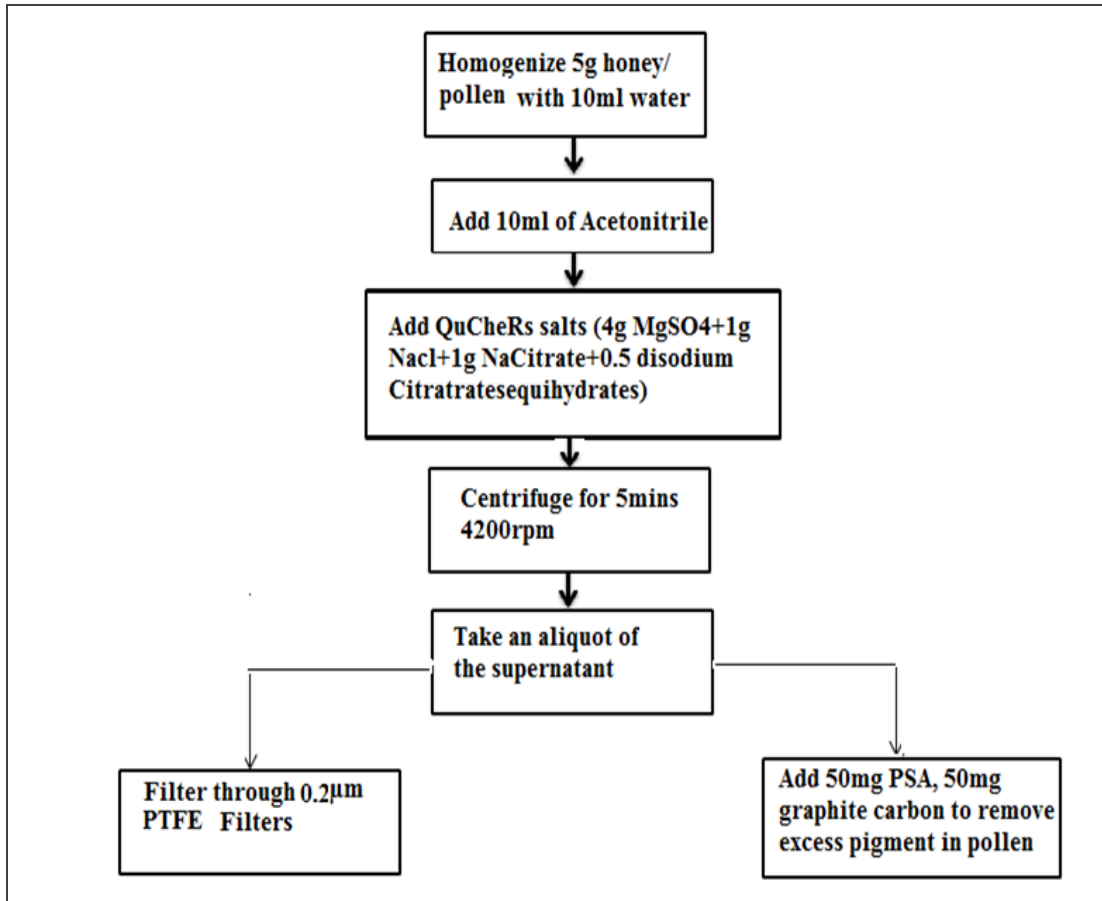


Figure 3.3 Sample preparation following QuEChERs method (Anastassiades *et al.*, 2003).

3.4.2 Extraction of Pollen

A 5 grams pollen (bee bread) sample was weighed into 50ml falcon tube separately and one blank pollen spiked with 0.1 µg/kg of neonicotinoids and prepared along with the samples for recovery evaluation as a control. A 10ml of LC-MS/MS grade water was added and the mixture was shaken for one minute followed by 10ml acetonitrile and vortexed for one minute again. A mixture of salts (4g of magnesium sulphate, 1g sodium chloride, 1g trisodium citrate dehydrate and 0.5g of disodium hydrogen citrate sesquihydrate) was added to induce phase separation. Pollen mixture was vortexed

for one minute and then centrifuged at 4,200 rounds per minute (rpm) for 10 minutes at 4°C. A 1ml of supernatant acetonitrile layer containing the neonicotinoid pesticides was drawn into 2.0ml eppendorf tubes and 150mg Magnesium sulphate, 50mg Primary Secondary Amine, 50mg Graphite Carbon (pre-packed) was added to remove polar pigment that may interfere with results and the final solutions was filtered through 2µm filter membrane then transferred to an auto sampler vial (1.5ml) for pesticide analysis using LC-MS/MS. (Plate 3.2 **A** and **B**)



Plate 3.2: Sample preparation and analysis in ICIPE Laboratory

3.5 Residue Separation using LC-MS/MS System

Liquid chromatography tandem mass spectrometry (LC/MS-MS) has analytical capability for monitoring systemic insecticides like neonicotinoids. The LC-MS/MS allows measurement of residues at parts per billion (ppb) (Plate 3.3). This analytical technique provides both qualitative (retention time and mass spectra) and quantitative

information. The combined technique allows all the various pesticides to be unambiguously identified in parts per billion concentration level.



Plate 3.3: LC-MS/MS Equipment used for neonicotinoid residue separation

3.5.1 Stock and Working Standard Solutions

Standard solutions were prepared at eight different concentrations of each pesticide; 0.05, 0.1, 0.5, 1.0, 5.0, 10, 25 and 50 parts per billion (ppb) and a blank (clean/reference sample) respectively. All pesticide standards were of high purity (>99% and were obtained from Sigma-Aldrich, Germany and were stored according to manufacturer's guidelines and specifications. Pesticide stock solutions were prepared in acetonitrile at 1 μ g/ml and stored in vials at -20 °C.

3.5.2 Calibration of Instruments

To determine the instrument signal response to changes in concentration, calibrations was done using working standard solutions of known and increasing concentrations for each analyte pesticides. By measuring the signals of the working standards, the LC-MS/MS constructs suitable calibration curves of response verses concentrations. The LC-MS/MS uses this suitable graph to determine concentrations of unknown analyte. The limit of quantification (LOQ) and limits of detection (LOD) was determined in accordance with (SANCO, 2013) protocol.

The detection limit of reporting pesticides in this study was based on the lowest limit of quantification that could give a spike recovery of >70%). In this case the limit of quantification was at 0.1ppb. The LC-MS/MS system's linearity was evaluated by assessing the signal responses of the calibration standards. The quantification of the identified pesticides was done by comparing the peak area of the analyte of the unknown concentration with those of the reference standards of known concentrations run at the same analytical conditions with samples (Janet *et al.*, 2016).

3.5.3 LC-MS/MS Operating Conditions

Samples were injected onto a C-18, 150 x 2.1mm 1.8 μ m, and Rapid Resolution reversed-phase column. A portion of 5 μ l of the sample was injected and the separations were performed at 35°C. The analytes were eluted from the column with a gradient flow (0.4ml/min) of 0.1% formic acid plus 5mmol ammonium formate in 100% water (mobile phase A) and 0.1% formic acid plus 5mmol ammonium formate in methanol (mobile phase B).(Table 3.1)

The gradient was held at 90% mobile phase A for one minute, before being ramped down to 20% over 9 minutes. This condition for elution was held for 10 minutes. The gradient was set back to initial conditions, and re-equilibrated for four minutes in preparation for the next sample injection. For the targeted analysis of neonicotinoids, the equipment was the same but specific scan events were used.

Table 3.1: LC-MS/MS operating conditions

Element	Characteristic features
Model	1290UHPLC series coupled to a 6490 model triple quadrupole mass spectrometer(Agilent technologies) for ion detection
Software features	Masshunter, enabled separating of co-eluting substances of different masses
Nebulizer gas and collision	Nitrogen gas
Mobile Phase A	100% water in 5mmol ammonium formate containing 0.1% formic acid in water
Mobile Phase B	Acetonitrile in 5mmol ammonium formate containing 0.1% formic acid in methanol
A gradient elution flow	0.4mL/minute
Sample Injection column	Rapid Resolution Reverse phase- C-18 , 150 x 2.1mm 1.8µm for high selectivity for most pesticides
Capillary voltage	3,500V
Nozzle voltage	300V
Limit of Detection(LOD)	0.05ppb
Limit of quantification (LOQ)	0.1ppb
ifunnel pressure	High pressure, RF 150V and low pressure RF 60V
Capillary Temperature	375°C at a spray voltage of 4.0kV
Ion source	Positive ESI-mode (electrospray ionisation)
Scan Type	Digital Multiple Reaction Monitoring

3.5.4 Working Principles of LC-MS/MS: 6490 Triple Quadrupole

LC/MS is hyphenated technique, which combined the separation power of High Performance Liquid Chromatography (HPLC), with the detection power of mass spectrometry. In this study 6490 Triple Quadrupole was used, it employs state of art of enhancements which include, electrospray ion source (1) and iFunnel technology (2) to improve on sensitivity via thermal gradient focusing and to enhance desolvation. The third enhancement is curved collision cell assembly filled with nitrogen collision gas (3) for creating ion fragments and the fourth enhancement is Quadrupole drive electronics (4) to improve on drive frequency resulting in higher mass resolution (Figure 3.4)

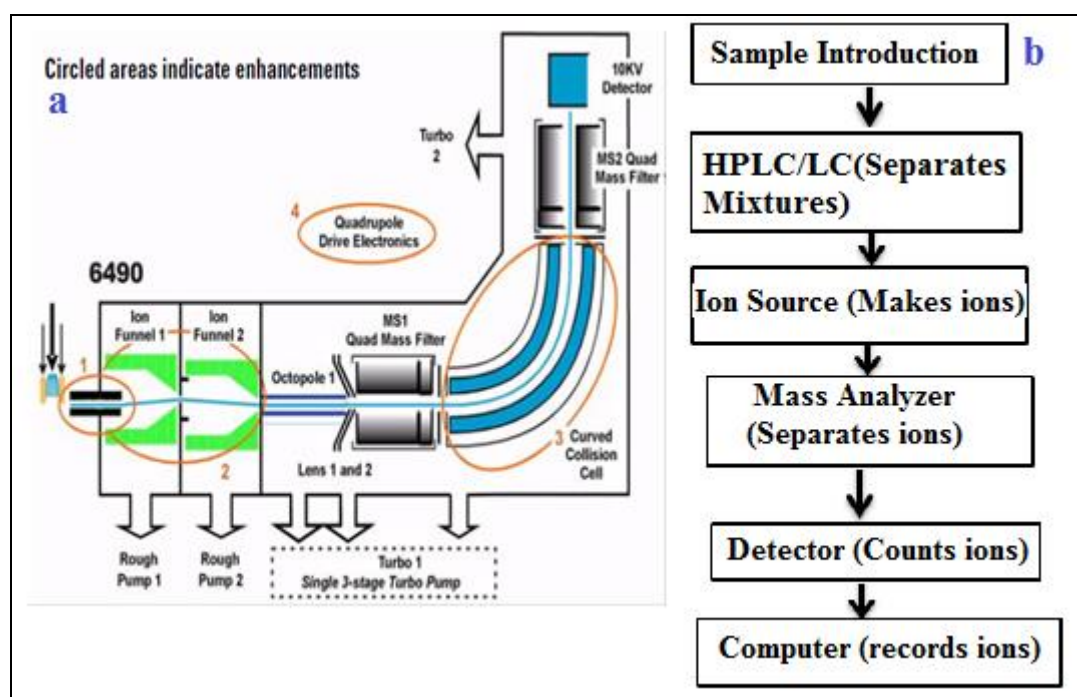


Figure 3.4 (a) Cross-section of LC-MS/MS Equipment used for neonicotinoid residue separation

(b) Schematic diagram representing LC-MS/MS sample analysis

Separation of sample component is achieved in HPLC column where the analytes are differently partition between the mobile phase and stationary phase. The separated sample species are then sprayed into ESI source where they are converted into ions in the gas phase. iFunnel requires levels of vacuum (rough vacuum) to operate efficiently. The octopole captures most of majority of ions from the source and direct to analyzer. The ions then move into first quadrupole analyzer (MS1) consisting of parallel hyperbolic rods through which selected ions based on their mass to charge ratio (m/z) are filtered.

The quadrupole mass analyzer consists of four parallel rods to which specific DC and RF voltages are applied. Analyzer require high vacuum and it uses three stage turbo pump. All ions (+, - and neutrals) that comprise the sample are generated at the source. However, when a specific set of voltages are applied, only ions of the corresponding m/z value may pass through the quadrupole to reach the detector. As the voltages are increased to other values, ions with m/z values are allowed to pass through. A full MS scan is obtained by increasing the DC and RF voltages applied to the four rods over and expanded range of values. These rods filter out all ions except those of one or more particular m/z values as determined by the voltages applied.

The ions passing through the first quadrupole analyzer are then directed through collision cell filled with pure nitrogen gas where they are fragmented. Fragments formed in collision cell are then sent to MS2 for second filtration stage to enable user to isolate and examine product ions with respect to precursor ions. Finally, the ions that pass through third quadrupole are detected using high energy detector. A second turbo pump has been added to increase pumping speed and to improve the vacuum

which will further improve on signal to noise ratio and enhance the limit of detection of triple quadrupole.

The collected data in this study was acquired through DMRM scan type which has a single continuous time segment and can end up in 4000 transitions in the scan segment table. At run time, pesticide transitions were automatically separated into multiple MRM tables according to the retention window of each transition.

3.6 Data Analysis and Presentation

Data obtained from questionnaires on pesticide usage and frequency of application were summarized into frequency counts and percentages in Ms Excel .Wilcoxon rank test was used to rank types of pesticides used in the region. Chi-square was used to compare frequency of pesticide use and applications in the studied areas. T-test was used to perform a comparison between concentration of residues detected in (i) honey (n=9) and pollen (n=9) and (ii) in different landscape structures (forested vs cultivated) and making comparisons with EU MRL in food products and significance differences accepted at $p \geq 0.05$ (Zar,2001) . The analysed data was then presented using bar charts, graphs, tables and boxplots.

CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.1 Introduction

The chapter presents the findings on socio-economic characteristics of bee keepers, the types of pesticides, their application frequency on cultivated crops around apiaries, neonicotinoid residue concentration levels in hive products (honey and beebread) from apiaries located in forested and cultivated landscapes in Kiambu and Nairobi County and lastly, results are discussed and compared with other similar studies.

4.1.1 Beekeepers' Socioeconomic Characteristics

The study revealed that out of the 72 beekeepers interviewed, 69% were male and 3% were female (Table 4.1). Majority of bee keepers were men due to the traditional believe that bee keeping is a man's activity and women are not allowed to venture into the activity. It is a taboo for women to harvest honey and therefore, the few women that are involved are required to employ men to undertake most of the tasks ranging from hive construction, hanging of hives on trees and subsequently harvesting. The male predominance in bee keeping could change in the near future as most organizations are advocating for engendered bee keeping. However, although men dominate honey production and harvesting, women are mostly involved in value addition activities and marketing of honey, meaning that they are important actors in the value chain.

Concerning education level of bee keepers, a small percentage (9.7%) of the respondents had not received any education, 27.8% had acquired primary education while 55.6% had gone to secondary and 6.9% had attained tertiary level of education,

which included technical Colleges and Universities (Table 4.1). Traditional bee keeping does not require formal education as the skills is passed down informally from the older experienced beekeepers.

Table 4.1: Beekeepers' socioeconomic characteristics

Characteristics	Frequency	Percentage (N=72)
Gender		
Male	69	95.8
Female	3	4.2
		100
Marital Status		
Single	12	16.7
Monogamous	53	73.6
Polygamous	5	6.9
Widowed	1	1.4
Separated	1	1.4
		100.0
Educational level		
None	7	9.7
Primary	20	27.8
Secondary	40	55.6
Tertiary college	5	6.9
		100.0
Main occupation		
Informal employment	30	41.7
Formal Employment	12	16.7
Business/self-employment	30	41.7
	72	100.0

About 41.7% of the interviewed bee keepers are engaged in informal employment while the rest are in business or formal employment. Most of those in formal employment do not find time to engage in farming, in this case bee keeping. Furthermore, the perception of bee keeping as an activity meant for those who are unemployed limits the engagement of the formally educated in this economic activity. Therefore this can be an important tool for employment creation in the area with high unemployment. About 80% of the bee keepers are married, while 16.7% are single. The rest are either widowed 1.4% or separated 1.4% (Table 4.1).

4.2 Types of Pesticides Applied and their Application Frequency on Cultivated Crops around Apiaries

4.2.1 Types of Pesticide applied on cultivated crops in the study area during the study period

The study revealed that the types of pesticides used on cultivated crops around the apiaries varied during the study period (Table 4.2). The pesticides used can be categorized into 7 classes; carbamates, organophosphates, herbicides, acaricides, neonicotinoids, pyrethroids and fungicides (Table 4.2). Carbamate based pesticides was the most applied and was applied by 32.4% respondents. The other pesticides applied were pyrethroids by 14.6%, neonicotinoids 14.4%, organophosphates 14.5%, herbicides 15.7%, acaricides 5.6% and fungicides by 1.4% of the respondents (Table 4.2). The pesticides were applied mainly on coffee, maize, vegetables, tomatoes and potatoes (Table 4.2).

The study also showed that of the 32.4% of the respondents who used carbamate based pesticides, 26.4% use carbaryl on fruits, 13.2% use karate (pyrethroids) on tomatoes, thiamethoxam (neonicotinoids) was used by 12% of the respondents on

coffee and roses, while dichlorvos (organophosphates) was used by 7.4% of the respondents on potatoes, 1.4% of the respondents did not use any pesticides at all (Table 4.2).

Table 4.2: Types of Pesticide used and applied on cultivated crops in the study area during the study period.

Trade name of pesticide	Classification	Crops grown near apiaries	% of respondent (N=72)
Cabaryl	Carbamate	Fruits	26.4
Karate	Pyrethroid	Tomatoes	13.2
Dimethoate (banned)	Organophosphate	potatoes	1.9
Thiamethoxam (actara)	Neonicotinoid	Coffee/horticulture	12
Metribuzin	Herbicide	Coffee	8
Pyraclostrobin	Organophosphate	Coffee/roses	1.9
Dichlorvos	Organophosphate	Potatoes	7.4
Atrazine	Herbicide	Coffee	1.9
Cypermethrin	Acaricide	Tomatoes/potatoes	5.6
Carbendazim	Carbamates	Roses	0.9
Mancozeb	Fungicides	Vegetables	0.9
Aldicarb (banned)	Carbamate	Maize	3.2
Bulldock	Pyrethroid/organophosphate	Tomatoes	0.5
imidacloprid(Thunder)	Neonicotinoids	Coffee/horticulture/Maize	1.9
Acetamiprid	Neonicotinoids	Coffee/horticulture	0.5
Ridomil	Carbamate	Tomatoes	0.9
Hexaconazole	Fungicide	Coffee/fruits	0.5
Bestox	Pyrethroid	Tomatoes	0.9
Antracol	Carbamate	Tomatoes	0.5
Diazinon	Organophosphate		0.5
Dithane M45	Carbamate	Tomatoes/vegetables	0.5
Malathion	Organophosphate	Coffee	2.8
Linuron	Herbicides	Maize	5.8
No pesticide use	-	-	1.4
			100

The study also revealed that banned or restricted pesticides are used in the study area.

The banned carbamate (aldicarb) and organophosphate (dimethoate) is used by 3.2%

and 1.9% of the respondents respectively while 1.9% of the respondents use the restricted thunder (imidacloprid) and 12% actara (thiamethoxam).(Table 4.3)

Table 4.3: Classification of the Common pesticide applied on cultivated crops in the area of study (April-August, 2015)

S/No	Pesticides Classification	Name of Pesticide	Type of Crop Grown	WHO Classification	Respondents (%) N=72
1.	Carbamate	Carbaryl	Fruits	Class IV	26.4
		Carbendazim	Roses	Class IV	0.9
		Aldicarb(Banned)	Maize	Class 1A	3.2
		Ridomil	Tomatoes	Class II	0.9
		Anthracol	Tomatoes	Class IV	0.5
		Dithane	Tomatoes	Class IV	0.5
		M45			
2.	Pyrethroids	Karate	Tomatoes	Class II	13.2
		Buldock	Tomatoes	Class II	0.5
		Bestox	Tomatoes	Class II	0.9
					14.6
3	Organophosphates	Dimethoate(Banned)	Potatoes	Class IB	1.9
			Coffee/rozes	Class II	1.9
		Pyraclostrobin	Potatoes	Class 1A	7.4
		Dichlorvos	Coffee	Class II	0.5
		Diazinon Malathion		Class III	2.8
					14.5
4	Herbicides	Metribuzin	Coffee	Class IV	8
		Atrazine	Coffee	Class IV	1.9
		Linuron	Maize	Class IV	5.8
					15.7
5	Neonicotinoids (Restricted)	Thiamethoxam	Coffee/horticulture	Class II	12
		Imidacloprid	Iture	Class II	1.9
		Acetamiprid	Coffee/horticulture/maize	Class II	1.9
			Coffee/horticulture		
					14.4
6	Fungicides	Mancozeb	Vegetables	Class IV	0.9
		Hexaconazole	Hexaconazole	Class IV	0.5
					1.4
7	Acaricide	Cypermethrine(Banned)	Tomatoes/potatoes	Class 1A	5.6
8	No pesticide use	-	-		1.4

KEY

Class IA – Extremely hazardous
Moderately hazardous

Class III – Slightly hazardous

Class IB – Highly hazardous Class II –

Class IV – Unlikely to present acute hazard

In Kenya the commonly used chemicals to control thrips, cutworms and aphids from vegetables are bestox, thunder (imidacloprid) dimethoate and bulldock (Gitonga *et al.*, 2011). However (Ngowi *et al.*, 2007) found that herbicides are rarely used in vegetable farming, while in Ghana herbicides are mostly used in vegetable farming (Ntow *et al.*, 2006). Study by Miriti, (2011) in Central Kenya found out that Linuron (herbicides) and diazinon (organophosphate) recorded high levels of application percentage. Pyrethroids and organophosphates are preferred by farmers due to their familiarity, different size packages and are affordable. Pyrethroids are also used most because they are low in toxicity to humans, mammals and birds (Tomlin, 1994).

According to WHO (2009) classification, dichlorvos (Phosvit) is extremely hazardous and is applied by small percentage of farmers. Macharia *et al.*, (2009) found out that carbamates are applied by 41%, followed by pyrethroids (19%), organophosphates (16%) and inorganics (5%). Carbamates are used widely because they are safer to humans, and are effective on crop pests (WHO, 1986).

Studies conducted by Lekei (2014), Kimani and Mwathi (1995), showed that labeling and packaging of pesticides in developing countries are often inadequate and inappropriate for the area where they are used. The advice is often written in a language that the user does not understand and the toxicity is explained poorly or not at all. In addition, the appropriate uses of the pesticide are usually not stated clearly and the dosage not specified. Yet, guidelines on good labeling practices have been published by FAO.

Studies done elsewhere (Tomizawa 2004; Mothiro 2004; Hopwood *et al.*, 2012; WHO, 1992) indicated that neonicotinoids are used because of their broad spectrum

activity, low application rates, low mammalian toxicity, target specificity, upward systemic movement in plants and versatile applications. However they are restricted because of high toxicity to pollinators.

Another study done around Lake Naivasha basin showed use of banned or restricted pesticides like endosulfan under various trade names (Njogu,2011).Other studies have shown build-up of pesticides in the food chain and some levels of contamination of water, sediments, eggs, crops and human fluid by pesticides (Njogu, 2011; Wandiga, 2001).

Study by Pradyot (2007) revealed that due to common pests attacking both vegetables and fruits including coffee, carbamate pesticides were used on both the vegetation and fruits. Another study by Masaya *et al.*, (2011) indicated that in underdeveloped and developing countries, the least expensive pesticides are utilized due to inability of the farmers to purchase more expensive safer products.

Other studies carried out elsewhere in Kenya (Liu *et al.*, 2012 ; Fishel, 2008) revealed that carbamates and pyrethroids are safer pesticides but are quite toxic to bees and parasitic wasps and their persistent use on plants pollinated by bees, could significantly reduce honey yields.

Another study done by Lichtenberg (2013) in Kenya revealed that the proximity of Kiambu to the capital city of Kenya, Nairobi creates a high demand for vegetable produce pushing farmers to harvest their crops before the required withdraw period and hence increased use of pesticides.

The study revealed that carbamates are widely used in the study areas; this could be attributed to them being less toxic to humans, availability on the shelves, effectiveness

and the fact that they are cheap. These pesticides are sold in small packages and weighed according to the amount required by respondents.

The continued use of banned pesticides could be due to availability of the pesticides on the shelves and the fact that they are quite effective. Use of carbamates and pyrethroid pesticides could have adverse effects on bees' pollinated crops. Consequently this could result in reduced yield in cross pollinated crops.

In 2012, the government of Kenya banned the use of dimethoate on fruits and vegetables for both export and the local market (GOK, 2012). Dimethoate was banned because it is carcinogenic while aldicarb can kill beneficial soil organisms. The restricted neonicotinoids included the highly toxic thiamethoxam (actara) and imidacloprid (thunder).

According to the pesticide Control Products Board of Kenya (PCPB). The national pesticide regulating authority in Kenya, dimethoate and other restricted pesticides are allowed into the country for restrictive use, only by informed users. However, the study showed that these pesticides are sold freely over the counter in many veterinary shops without any proper monitoring or adherence to the stipulated restrictions on their sale and application and therefore their presence in the Kenyan market presents a great risk to both environment and users. Although pesticide regulatory mechanisms exist, their weak structures enabled the importation and usage of pesticides banned in the country of origin.

More pesticides are applied on crops like tomatoes for local and other markets due to sensitivity of the crop to pest- invasion and abrupt changes in temperature and other agrochemicals. Some respondents used more than one pesticide in one application. It

is evident that many farmers cannot grow crops without use of pesticides. Farmers use same pesticides to different crops due to their availability and also lack of finance to buy the other pesticides like pyrethroids.

Most of the insecticides belong to pyrethroid group and organophosphorus group. The popularity of the two could be attributed to perceived effectiveness and their being in the market for a long time. Usually farmers buy what was recommended from the various information sources and tend to stick to familiar pesticide names over time. Pyrethroids and organophosphorus pesticides came in different size packages and the farmers can choose the quantity to purchase usually depending on experience, affordability and crop population.

Poverty affects the pesticide problem in variety of ways. An illiterate farmer is unable to read the directions about proper pesticide use. Financial situations do not allow them to purchase the adequate spraying equipment or safer, but more expensive pesticides.

Wide use of actara (thiamethoxam) and imidacloprid could pose great danger to birds which feed on insects especially in agricultural areas where neonicotinoids are used extensively. Thiamethoxam and imidacloprid is used to coat seeds of maize. Consumption of small numbers of dressed seeds offers a potential threat to granivorous birds.

The study sites supports a large horticultural industry both for export and domestic markets and this has consequently resulted into increased use of pesticides. Major crops grown in the study sites included; french beans, peas production for the export

market, pineapple production as well as coffee-growing all these depend heavily on pesticide use hence health risks to honey bees

4.2.2 Frequency of Pesticides Application on Cultivated Crops Around the Apiaries During the Study Period

As evidenced in Figure 4.1, the frequency of pesticides application in the study area varied widely during the study period. The study showed that 86.1% of the respondents apply pesticides on weekly basis while 12.5% do it fortnightly and 1.4% applies when they have money to buy the pesticides (Figure 4.1). Using Chi-square, there was no significant difference between the respondents who apply pesticides weekly and those who do fortnightly ($\chi^2 = 0$, $df= 1$, $n=2$, $p=1$) (Figure 4.1).

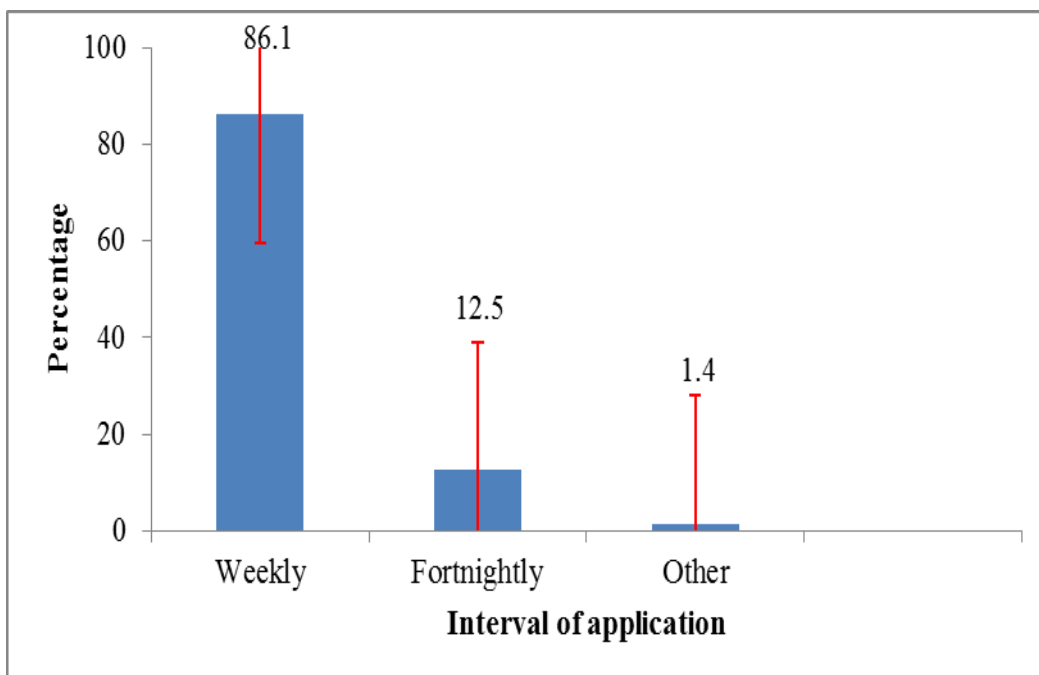


Figure 4.1: Frequency of pesticide use and application

According to Mondia *et al.*, (2003) and Williamson *et al.*, (2003), the frequency of pesticide application depends on the type of crops grown, economic status of farmers whether it is a plantation or small scale. The frequency of application is also determined by potency of the pesticides (Gitonga *et al.*, 2010; Ngowi, 2007; Dinham, 2003) or availability of the pesticide and season of the year (Miriti, 2011).

The high number of respondents who apply pesticide weekly in the study area could be attributed to economic status where most of the respondents had informal employment and poor thus they could not afford the most expensive and effective pesticide brand, wet sampling season diluted the chemicals applied and thus farmers had to apply them more often and frequent to be effective. Availability of the pesticide on the shelves where the farmers could easily pick and use, these may prompt farmers to use same pesticides to different crops. The high frequency of pesticides application by the farmers is also attributed to the fact that farmers spray in anticipation of pests and disease outbreak and hence is a preventive measure. Mixed cropping also attracts many different pests and this may also influence farmers to use more than one pesticide in one application in order to control pests.

4.3 To Analyze Levels of Neonicotinoid Residues in Hive Products (Honey and Pollen)

4.3.1 Neonicotinoid Residue Concentrations in Honey

The study revealed that honey is contaminated with neonicotinoids residues. The concentration of neonicotinoids in the honey varied during the study period (Figure 4.2, Table 4.4). The neonicotinoids (acetamiprid) residue in honey ranged from 0.1ppb, limit of quantification in Lari, Gatundu, Ruiru, Thika, Kikuyu, Karura forest and Ngong forest to 0.5ppb in Thika IPM (Table 4.2). While the levels of other

neonicotinoids, thiamethoxam and imidacloprid were below the limit of quantification in all sampled apiaries during the study period (Table 4.4). The concentrations of all residues were below the EU recommended levels (thiamethoxam: 10ppb and imidacloprid: 50ppb and acetamiprid 50ppb).

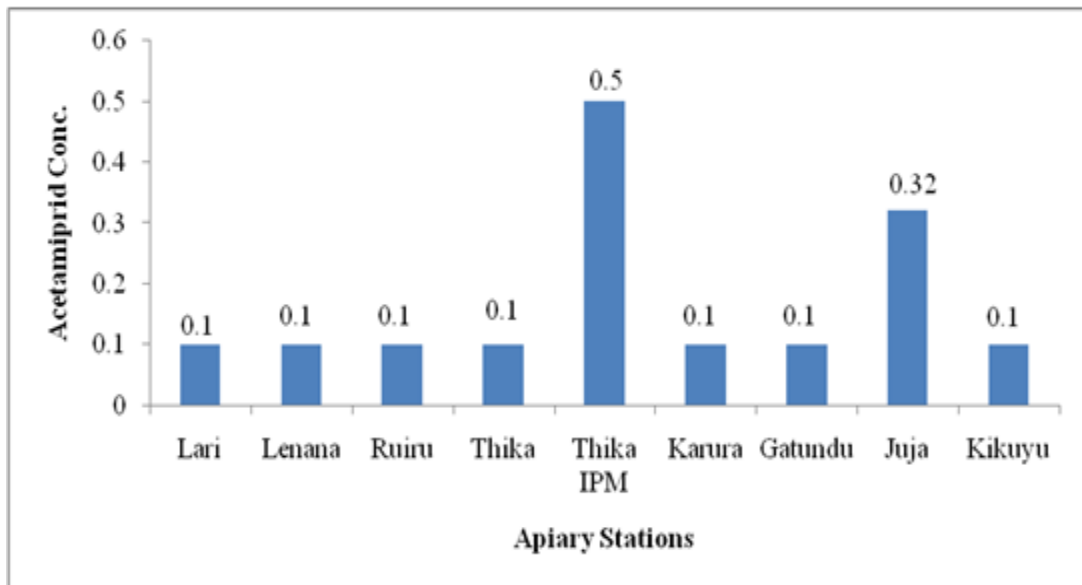


Figure 4.2: Levels of Acetamiprid in Honey samples

Table 4.4: Neonicotinoids compounds detected in honey from sampled apiary sites

A report of (< LOQ) means that no neonicotinoids were found with a concentration higher than the limit of quantification (LOQ)

Apiary/ Residue	Thiamethoxam (ppb)			Imidacloprid (ppb)			Acetamiprid (ppb)		
	Detection	EU standards	P Values	Detection	EU standards	p-Values	Detection	EU standards	p-Values
Karura	< LOQ	10	-	< LOQ	50	-	0.1	50	
Ngong	< LOQ	10	-	< LOQ	50	-	0.1	50	
Lari	< LOQ	10	-	< LOQ	50	-	0.1	50	
Ruiru	< LOQ	10	-	< LOQ	50	-	0.1	50	
Thika	< LOQ	10	-	< LOQ	50	-	0.1	50	
Thika IPM	< LOQ	10	-	< LOQ	50	-	0.5	50	
Gatundu	< LOQ	10	-	< LOQ	50	-	0.1	50	
Juja	< LOQ	10	-	< LOQ	50	-	0.32	50	
Kikuyu	< LOQ	10	-	< LOQ	50	-	0.1	50	0.001

Note: LOQ \geq 0.1 ppb \equiv below limit of quantification

Using T-test analysis, it showed that there was no significant difference ($t = 3.5206$, $df = 8$, $p\text{-value} = 0.007839$) in acetamiprid concentration levels in honey from Thika IPM and that of Juja apiaries (Table 4.4).

Studies in France indicate that pesticide residues found in honey and bee bread of beehive was not systematically present in another matrices such as bees wax and

pollen (Decourtye *et al.*, 2004). The concentration of pesticides in honey is influenced by distance of colonies from intensive conventional cropping/horticulture (roses and coffee farms), frequency of application, time and season of treatment (meteorological condition during application), nature of surroundings (Krupke *et al.*, 2012; Henry *et al.*, 2012; Halm *et al.*, 2006; Rortais *et al.*, 2005). It is also influenced by specific bio-transformations of residues in honey and pollen (Bonmatin, 2005). During transformation process from pollen to honey most pesticides degrade and lost, toxic nature of the pesticides ((EU pesticide database, 2011; Muli *et al.*, 2014) and behaviour of bees as natural filters, bees have catholic tastes and are capable to weaken the pesticides in honey and lethal dose exposed during bee foraging activities (Thompson *et al.*, 2013; Klein *et al.*, 2012; Bogdanov *et al.*, 2003).

The presence of acetamiprid in honey products in Thika IPM and Juja could be attributed to the fact that apiaries in Thika IPM were in close proximity to roses and those in Juja were close to coffee farms. Farmers in these apiary sites indicated versatile and wide application of acetamiprid to control pests on the respective crops.

4.3.2 Neonicotinoid Residue Concentration in Pollen

The study revealed that the concentration of neonicotinoids in pollen varied during the study period. The concentration of neonicotinoids thiamethoxam varied from below the limit of quantification in Ngong to 47.80 ppb in Thika IPM, imidacloprid varied from below the limit of quantification to 2.19 ppb in Thika IPM, while acetamiprid was below the limit of quantification in all the samples apiaries (Table 4.5 and Figure 4.3). High concentration of thiamethoxam, 47.8 ppb and imidacloprid was recorded in apiaries found in Thika IPM.

Table 4.5: Neonicotinoid detected in pollen (bee bread) and their concentration levels in ppb at 9 apiary sites

Apiary/ Residue	Thiamethoxam (ppb)			Imidacloprid (ppb)			Acetamiprid (ppb)		
	Detection	EU standards	P Values	Detection	EU standards	p-Values	Detection	EU standards	p-Values
Karura	0.71	10	-	0.10	50	-	<LOQ	50	-
Ngong	0.10	10	-	0.10	50	-	<LOQ	50	-
Lari	0.10	10	-	0.10	50	-	<LOQ	50	-
Ruiru	0.10	10	-	0.10	50	-	<LOQ	50	-
Thika	0.10	10	-	0.45	50	-	<LOQ	50	-
Thika IPM	47.80	10	-	2.19	50	-	<LOQ	50	-
Gatundu	0.10	10	-	0.10	50	-	<LOQ	50	-
Juja	0.10	10	-	0.43	50	-	<LOQ	50	-
Kikuyu	10.92	0.10	-	0.10	50	-	<LOQ	50	-

Note: LOQ ≥ 0.1 ppb \equiv below limit of quantification

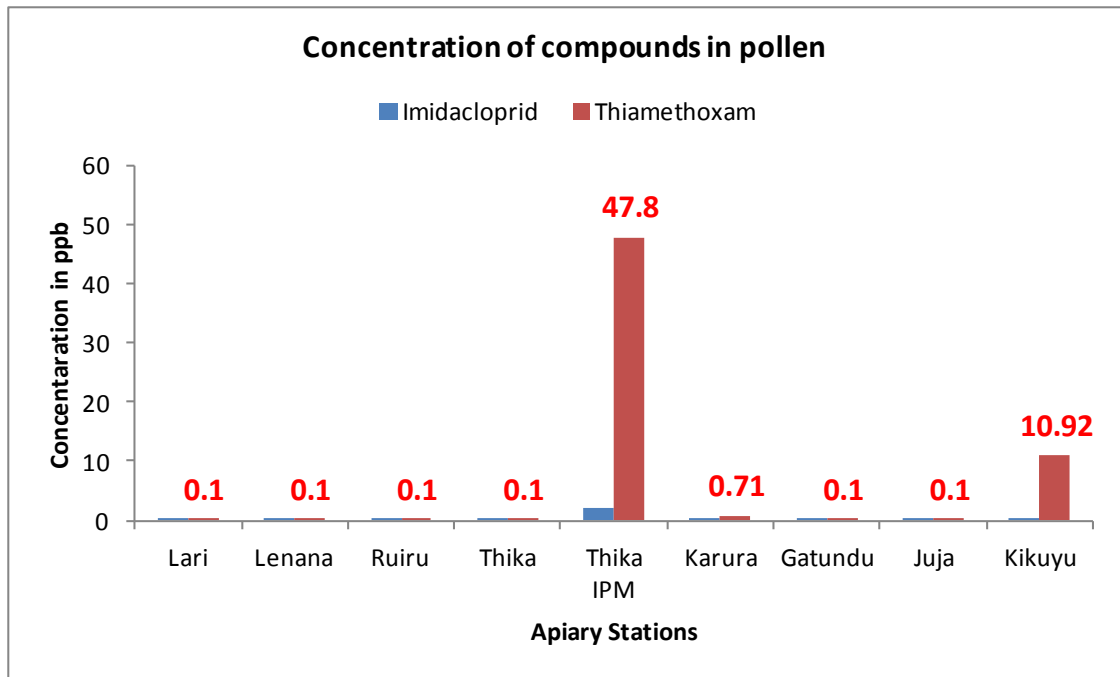


Figure 4.3: Mean Levels of neonicotinoids detected in pollen between April and August, 2015 in selected apiaries.

Using t-test analysis, it showed that there was no significance difference ($t = 1.3279$, $df = 1$, $p\text{-value} = 0.4109$) in mean concentrations of thiamethoxam in Karura and Ngong apiaries. The t-test analysis also failed to show significant difference in mean concentrations between Imidacloprid concentration in Thika and Thika IPM apiaries ($t = 1.5172$, $df = 1$, $p\text{-value} = 0.371$).

High concentration of thiamethoxam was detected in the pollen from Thika IPM. The other apiary where thiamethoxam was detected was in Karura forest (0.71 ppb) and Kikuyu (10.92 ppb). Imidacloprid was also detected in higher concentrations in Thika and Juja apiaries: 0.45 and 0.43 ppb respectively but were below the EU levels (Table 4.5).

Since the levels of concentration fall below the alarm threshold levels of 50 ppb, it could be suggested that beekeepers in the studied counties may still use the current pesticides, but should monitor and control the levels so that toxicity levels do not exceed the maximum residue limits (MRL) which could cause health problems.

The presence of different concentrations of neonicotinoids in pollen depends on high application and sampling methods at a single point (UNEP; 2010; Krupke, 2012; Hopwood *et al.*, 2012). The type of crops treated with the chemical around apiaries have been found to influence the presence of neonicotinoids in pollen (Williams *et al.*, 2010; Kievits *et al.*, 2007; Rortais *et al.*; 2005 ; Decourtye *et al.*, 2004; Halm *et al.*, 2006).

The concentration levels in pollen depend on the contamination of bee bread through contact and oral means (Bonmatiet *et al.*, 2005), the size of plantation/ crops grown around the apiary, season of application and the time the bees feed on stocked pollen for food resources (Lambert *et al.*; 2012; Hopwood *et al.*; 2012 and Nguyen *et al.*, 2009).

The presence of thiamethoxam in high concentration might be reflecting changes that are happening in agricultural practices where imidacloprid is being replaced by new neonicotinoids such as thiamethoxam in the market. Thiamethoxam was detected at higher concentration (0.04780mg/kg) at Thika IPM in this study. The Maximum Residue Limits (MRL) set for this compound in honey is 0.01mg/kg and is considered to be highly toxic to honey bees with LD₅₀ of 0.025-0.029µg/bee (Table. 4.6). Its presence in pollen could be attributed to a high application of the compound in the field; the honey bees collected the fresh contaminated pollen during foraging

activities and transported thiamethoxam into the beehives through contact and oral exposure.

Table 4.6: Maximum Residue Limits for Neonicotinoid Insecticides

Substance Residue	MRL Honey($\mu\text{g} / \text{kg}$)	in Oral Toxicity	Contact Toxicity
Acetamiprid	50	$\text{LD}_{50}=14.53 \mu\text{g} / \text{kg}$	$\text{LD}_{50}=8.09 \mu\text{g} / \text{kg}$
Imidacloprid	50	$\text{LD}_{50}=0.081 \mu\text{g} / \text{kg}$	$\text{LD}_{50}=0.243 \mu\text{g} / \text{kg}$
Thiamethoxam	10	$\text{LD}_{50}=0.005 \mu\text{g} / \text{kg}$	$\text{LD}_{50}=0.029 \mu\text{g} / \text{kg}$

Key: $1 \mu\text{g} / \text{kg} = 1\text{ppb}$ (Source: European Union, 2011)

Thika IPM was the only apiary site where both small scale and large scale farming of horticultural, French beans and coffee was practiced intensively. The colonies within the apiary were located very close to flower farms. Wet season sampling at the site coincided with periods of heavy pesticide spraying and application and high foraging activity. Most farmers in the site indicated that they used actara/ thiamethoxam in their farms to control pests in their coffee and horticultural farms

4.4 Neonicotinoid Concentrations in Cultivated and forested Landscapes

The concentration of neonicotinoids in apiaries located in cultivated and forested landscapes showed variations during the study period. The concentration of neonicotinoids ranged from below the limit of quantification to 47.80 ppb (thiamethoxam) in ThikaIPM. Thiamethoxam was the only neonicotinoids whose concentration was above the limit quantification in Thika IPM and in Kikuyu apiaries (Figure 4.4 and Figure 4.6) and acetamiprid was below limit of quantification in all sampled sites while imidacloprid was detected in Thika.

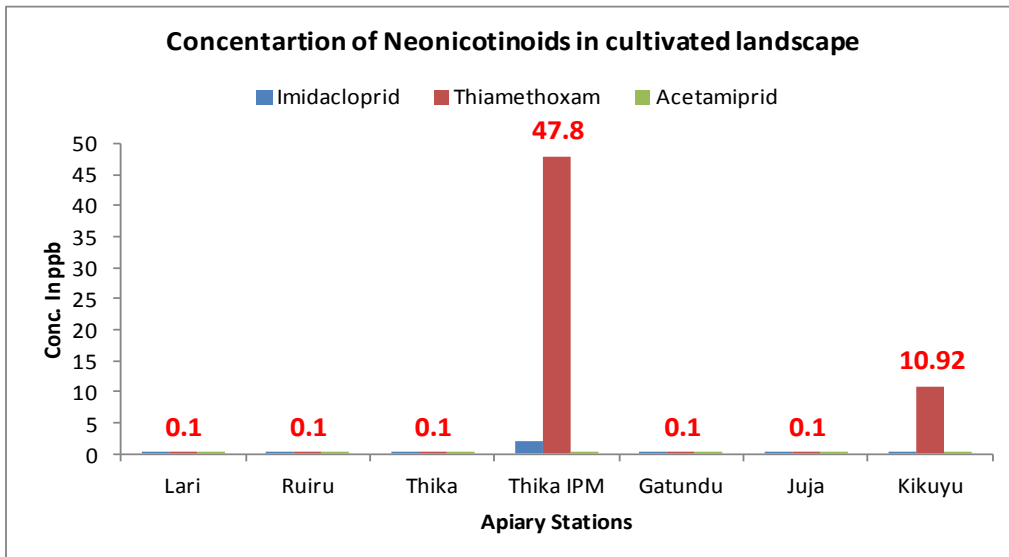


Figure 4.4: Concentration of Neonicotinoids in cultivated landscape.

In forested landscape only thiamethoxam was above limit of quantification (Figure 4.5).

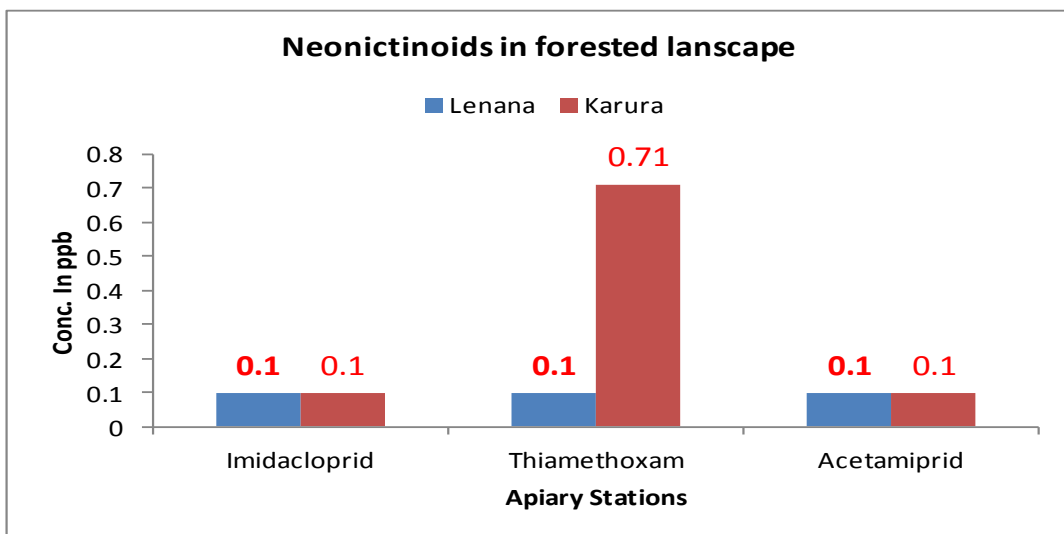


Figure 4.5: Concentration of neonicotinoids in forested landscape. Study Conducted: April, 2015 – August, 2015

Using a two sample t-test at 95% confidence level, showed significant difference in concentration of thiamethoxam for both cultivated and forested ($p=0.009998$).

Contamination of hive products in different landscapes, countryside, urban, forested and cultivated landscapes depends on the type of landscape exposed to the pesticide pressure. Countryside landscape are more contaminated than urban landscapes, cultivated landscapes are more contaminated than forested landscapes (Perugin *et al.*, 2011). Contamination levels also depend on the location of the apiaries. Apiaries located in agricultural fields with intensive monocultures and high anthropogenic activities are more contaminated with pesticides due to wide and versatile pesticide use compared to apiaries located in forested landscapes which are more natural (Krupke *et al.*, 2012). Concentration of neonicotinoid also depends on the prevailing meteorological condition during sampling stage (Krupke *et al.*, 2012; Ghini *et al.*, 2004).

The difference in contamination levels in forested and cultivated landscapes could be due to the different agronomic activities found in these studied areas. For instance, cultivated landscapes were subjected to high agronomic activities and thus were more contaminated whereas forested landscapes were less contaminated due to limited agronomic activities. However, forested landscapes showed moderate levels of thiamethoxam and this could be attributed to wide application of the chemical in raising nursery seeds before transplanting.

Cultivated landscape also revealed possibilities of hives being susceptible to various contamination levels due to varied uses of the pesticides in home gardening and horticultural practices. Thiamethoxam is widely applied in the coffee and flower farms in close proximity to the sampled colonies. The cultivated landscapes were dominated by permanent heavy pesticide dependent crops such as; roses, vegetables

French beans, and coffee plantation. Wet season sampling coincided with periods of heavy pesticide spraying and application and high foraging activity.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

This chapter provides conclusions and recommendations based on the objectives and findings of the study. The general objective of the present study was to identify and quantify neonicotinoid residues in hive products in selected apiaries in Kiambu and Nairobi Counties.

5.2 Conclusions

1. The types of pesticides applied on commonly cultivated crops around the apiaries were carbamates (32.4%), pyrethroids (14.6%), neonicotinoids (14.4%), and organophosphates (14.5%). Other pesticides used were fungicides, herbicides and acaricides.
2. The highest frequency of pesticide application was weekly (86.1%) while 12.5% applied fortnightly and 1.4% applied when money is available.
3. Neonicotinoids residues, acetamiprid, imidacloprid and thiamethoxam were found in hive products (pollen and honey). Acetamiprid was the only pesticide detected in honey at levels below its acceptable Maximum Residue Limits.
4. There were higher concentrations of neonicotinoids in hive products located in cultivated areas. Thiamethoxam was detected at the highest concentration in both regions with a maximum concentration of 0.71 ppb in forested areas and 1.02 ppb in cultivated landscapes.

5.3 Recommendations

1. Strict measures should be put in place and enforced by regulatory authorities like NEMA, KEPHIS, PCPB and Ministry of Agriculture so that banned pesticides are not easily available to farmers.
2. Farmers should be advised on environmentally safe methods of applying pesticides and on safe frequencies, weekly application may contaminate the food and fruits, these chemicals accumulate and persist in the environment.
3. Though levels of neonicotinoid pesticides detected were below the EU recommended levels in honey, pesticides are persistent and accumulate in the environment and their levels should be monitored regularly.
4. The apiaries should be located away from cultivated areas of intensive conventional cropping to minimize honey bees exposure by foraging on contaminated pollen and nectar and carrying pesticides into the food chain through the hive matrix and this will reduce neonicotinoids in hive products.

5.4 Further Research

1. Work on effects of neonicotinoids on African honey bees health should be done in different locations in the country, the focus should be on areas with depopulated beehives.
2. Additional studies should be conducted to evaluate pesticide residues in honey from our local markets, roadsides, shops and supermarkets to assure consumers safety and product purity. This is especially important for international trade and safeguarding the environment.
3. Other studies should be carried out to determine seasonality variations of neonicotinoid residue concentrations in hive products.

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APPENDICES

Appendix I: Research Authorization letter from NACOSTI



**NATIONAL COMMISSION FOR SCIENCE,
TECHNOLOGY AND INNOVATION**

Telephone: +254-20-2213471,
2241349, 310571, 2219420
Fax: +254-20-318245, 318249
Email: secretary@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No.

Date:

10th December, 2014

NACOSTI/P/14/4732/4269

Protus Wanjala Mulati
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research *“Impact of neonicotinoids on honey bees (Apis Mellifera) and their ecological services in selected apiaries in Kiambu County, Kenya,”* I am pleased to inform you that you have been authorized to undertake research in **Kiambu County** for a period ending **15th June, 2015**.

You are advised to report to **the County Commissioner and the County Director of Education, Kiambu County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.


DR. S. K. LANGAT, OGW
FOR: SECRETARY/CEO

Copy to:

The County Commissioner
Kiambu County.

The County Director of Education
Kiambu County.

Appendix II: Questionnaire for Beekeepers in Kiambu County.

My name is ProtusWanjalaMulati Master of Environmental Science student at Kenyatta University. I have cleared my course work and therefore am currently embarking on a research project, titled “**Evaluation of Neonicotinoid Residues in Hive Products from Kiambu and Nairobi Counties, Kenya**”

The study is intended to investigate the types of pesticides applied on cultivated crops and suggest appropriate conservation measures that can be put in place to protect honey bees from poisoning with pesticides. Information which will be obtained will be used for academic purposes only and will be treated with absolute confidentiality.

This questionnaire is meant to collect data for the research project. You have been selected as one of the respondents. Kindly provide your honest information on all the items in this questionnaire. It is purely for academic pursuit and the views expressed will be treated confidential.

INSTRUCTIONS

This questionnaire consists of **four (4) pages printed one side**. There are 13 questions in total please answer all questions to the best you can. **Respond by Ticking [√] or circling** your answer choice from options provided. Where **applicable explain or make your suggestions** on the spaces provided. **Note that the number values** on the possible option has no implication on any choice

Date _____ Time _____

Sub-County _____ Ward _____

Village _____

1. By use of a **tick [√]** or **circling** please indicate your gender [1] Female [2] Male

2. By use of a **tick [√]** or **circling** please indicate your age bracket?

[1] 18 - 25 years [2] 26 - 35 years [3] 36 - 45 year 46 – 55 years [4] above 56 years

3. By use of a **tick [√]** or **circling** please indicate your highest level of education

[1] No education [2] Primary school education [3] Secondary school education

[4] Others, please indicate _____

4. By use of a tick [√] identify cultivated crops sprayed with pesticides or any chemical substances in your farm

Crop	Yes	No
Coffee		
Commercial flowers(roses)		
Tea		
Ornamental flower plants		
Pineapples		
Tomatoes		

Crop	Yes	No
Cabbages		
French beans		
Maize		
Beans		
Others (specify)		

5. How often do you spray your crops

[1] Weekly [2] Fortnight [2] Other

6. Which pesticides or any chemical substances you spray crops with

i) _____

ii) _____

7. (a) Do you spray your hive with pesticides or any chemical substances to control any of the above pests? [1] Yes [2] No


(b) If YES in question 10(a) above which pesticides or any chemical substances do you apply?

i) _____

ii) _____

iii) _____

Appendix III: ICIPE Letter for Dissertation Internship Programme



icipe
African Insect Science for Food and Health

International Centre of Insect Physiology and Ecology
P. O. Box 30772-00100 Nairobi, Kenya
Tel: +254 20 8632000; Fax: +254 20 8632001/2
icipe@icipe.org — www.icipe.org

Our Ref: CBID/DRIPMSc/PM0515 25 May 2015

Protus Wanjala Mulati
P.O. Box 52428 00200
Nairobi, KENYA

Dear Mr. Mulati,

icipe's DISSERTATION RESEARCH INTERNSHIP PROGRAMME (DRIP)


Letter of Offer

I am pleased to make you an offer of research internship, to undertake research work towards an MSc degree to be awarded by Kenyatta University, at *icipe's* Duduville Campus, Nairobi, Kenya. This offer of internship is provided under *icipe's* Dissertation Research Internship Programme (DRIP). The following terms and conditions will apply during your research internship:


1. **Position:**
During the period of your training, you will hold the position of Graduate Research Intern, under *icipe's* DRIP Programme.
2. **Duration and Effective Date:**
The internship is for 6 months and takes effect from **1 May 2015** and will end on **31 October 2015**.
3. **Purpose:**
This research fellowship is provided specifically for an MSc research study titled "Impact of neonicotinoid residues on African Honey Bees" (*Apis mellifera scutellata*) health in selected apiaries in Kiambu County, Kenya" as specified by your academic and research supervisors. You will be expected to undertake field studies as part fulfillment of postgraduate studies at Kenyatta University within the context of the collaborative research project.
4. **Affiliation and Supervision:**
You will be hosted by the Bee Health Project at *icipe's* Duduville Headquarters, Nairobi. Your supervisors will be Dr. Janet Irungu (*icipe*) and your appointed supervisor from Kenyatta University. We encourage you to get in touch with Dr. Irungu (jirungu@icipe.org) who will provide you with details of your training and research programme.
5. **Training and Research facilities:**
You will have access to training facilities in the host department, inclusive of laboratory space and supervision. Your project will be hosted jointly by Kenyatta University where you are registered as a Masters' level student, in collaboration with *icipe's* Bee health Project, which will provide basic equipment and supervision of your research training in both field and laboratory aspects.
6. **Emoluments and Personal Support:**
 - a) You will receive a nominal transport allowance of Kenya Shillings 10,000 per month (ten thousand shillings only), payable in arrears, at the end of each month of your internship. This allowance will be charged to the Bee health Project budget code: B4124A-61000-004, and is for meeting your transport and commutation expenses to the centre.

Please note that it is your responsibility to comply with the income tax regulations and requirements of your home country. In view of this, *icipe* will deduct the applicable Pay as You Earn (PAYE) amounts from your transport allowance (*if applicable*), for onward submission to the Kenya Revenue Authority (KRA). The fellowship makes no extra provision for students with dependants.

Cont'd.../2



An FAO Reference Centre and a Stockholm Convention Regional Centre



Mr. Protus W. Mulati,
Kenya University

b) Medical, Personal Accident and Group Life Insurance Benefits:
icipe will not provide local medical insurance, group life and personal accident insurance cover during your internship, as you already have insurance cover under ICEA Lion General policy number 998-A8-524825-15, as confirmed by the letter issued by ICEA Lion General Insurance Ltd, dated 22 May 2015, and submitted to *icipe*'s CB&ID programme.

c) Training fees:
A Bench fee of US\$ 100 per month is levied to all MSc research fellows and interns visiting *icipe* for more than 4 weeks, and this is payable at the commencement of training. This fee is a contribution to the maintenance of scientific equipment, facilities and infrastructure that is available to the visiting researcher. With adequate justification, waivers are granted by *icipe*'s Board of Training and Postgraduate Studies (IBTPS). We are pleased to inform you that in your case these fees have been waived.

8. Progress Reports:

In accordance with the regulations of the registering university, you will be required to prepare a training report or approved thesis for examination, at the end of your research internship. You are also expected to make a seminar presentation on your research study and findings to the *icipe* scientific community at the end of your internship.

9. Intellectual Property, Publications and Reporting:

You will be expected to sign the *icipe* Intellectual Property Undertaking Form. We enclose a copy for this purpose.

If you publish research that has utilized *icipe*'s resources in the form of financial support from an *icipe*-led grant or *icipe* facilities then you must include *icipe* as your affiliated institution when authoring the publication. If you have a second host institution, for example a University in the case of a graduate student, then you can also list this affiliated institution. If you undertook the work whilst at *icipe* and leave before writing up the results for publication then you must list *icipe* as your affiliated institution for the purpose of the research, though you may also include your new address for correspondence. Also, *icipe* has a very clear policy relating to publication authorship. You must read and understand the policy, which is available at

http://intranet.icipe.org:8000/intranet/index.php?option=com_content&view=article&id=810%3Aicipe-publication-policy-march-2010&catid=29&Itemid=40

10. Staff rules and Regulations:

You will be expected to observe and adhere to the rules and regulations governing the conduct of staff at the Centre as well as the policies and conditions of postgraduate training at the Centre. This offer of training attachment may be withdrawn on account of breach of discipline.

If you accept the terms of offer contained herein, please return the provided form duly signed within the next ten (10) days.

Let me congratulate you on this offer and wish you the very best in your training attachment.

Yours sincerely,



Robert A. Skilton (PhD)

Head, Capacity Building & Institutional Development (CB&ID) Programme

cc:

- 1) *icipe* Supervisors: Dr. J. Irungu (Bee Health)
- 2) Human Resources Manager: Mr. W. Awori
- 3) HR Payroll & Benefits: Mr. K. Mutunga
- 4) Manager, Project Accounts: Mr. P. Ndirang'ui

Encl:

- i. DRIP Policy
- ii. Policy on Intellectual Property

Appendix IV: Bee surveillance consent form

International Centre of Insect Physiology and Ecology

Duduville Campus, Off Thika Road, Kasarani

P.O. Box 30772-00100, Nairobi, KENYA

Tel: +254 (20) 8632060, +254 (20) 8632000

16th May 2015,

Dear Beekeeper,

The International Centre of Insect Physiology and Ecology (*icipe*) is an intergovernmental organization, based in Nairobi Kenya, with a mandate to carry out research and development activities in environmentally sound and sustainable management of arthropods for improving health and agricultural productivity in the tropics. *icipe* is actively involved in capacity building programs at various levels (interns, postgraduate, postdoctoral and professional levels).

Besides producing honey, honeybees are regarded as the most important pollinators in the agricultural sector. Approximately 1/3 of all the plants we eat require pollination by bees. In the recent past, some beekeepers have experienced serious losses of honeybees due to diseases, pests and pesticides, which are of great concern, as such; colony losses represent a major threat to our food supply. In response to these observations, *icipe* in collaboration with Kenyatta University is currently conducting a one year study on bee health. This will enable us to gain knowledge about the prevailing pesticides in this county and offer sustainable solutions towards their management for food security.

In this regard, we ask you, as a beekeeper to partner with us by allowing us to monitor your colonies over the study period. *icip*e will endeavour to equip you with the knowledge and skills required to identify problems in your colonies and to collect samples and record information correctly.

If you are in agreement with our proposal, kindly fill out the attached consent form.

We look forward to working with you towards a sustainable understanding, care, and protection of African bees.

Thank you for your cooperation.

ProtusWanjalaMulati

Research Scientist, ICIPE

Appendix V: Consent Form for Participation in a Research Study on Bee Health

The International Centre of Insect Physiology and Ecology (*icipe*).

Researcher(s): ProtusWanjalaMulati)

Research Scientist, ICIPE

Study Title: Neonicotinoid Residues in African Honey Bees' (*Apis Mellifera Scutellata*) and Hive Products in Selected Apiaries in Kiambu County, Kenya

Funding Agency: Researcher

1. What is this form?

This form is called a Consent Form. It will give you information about why this study is being done and why you are being invited to participate. It will also describe what you will need to do to participate and any known risks or inconveniences that you may have while participating. We encourage you to ask questions now and at any other time. If you decide to participate, you will be asked to sign this form and you will be given a copy for your records.

2. Who is eligible to participate?

Any beekeeper with an apiary having at least 5 colonies is eligible to participate in this study.

3. What is the purpose of this study?

The purpose of this research study is to enable the researchers to gain knowledge about the prevailing pesticides in particular neonicotinoids and the effect of pesticides on honeybees in this region.

4. Where will the study take place and how long will it last?

The study will take place at the apiaries of willingly recruited beekeepers *Researcher* will monitor their colonies over an initial period of 1 year. The Researcher will visit your farm at least twice in a year.

5. What will I be asked to do?

If you agree to take part in this study, you will be asked to provide some information to the researchers by answering a structured questionnaire to provide information about your experience as a beekeeper. We will ask you information regarding your farm and the surroundings. The questions are not designed to invade on your privacy and you may skip any question you feel uncomfortable answering.

6. What are my benefits of being in this study?

You may not directly benefit from this research; however, we hope that your participation in the study may provide us with information that will enable you to improve your apiary management.

7. What are my risks of being in this study?

We believe that there are no known risks associated with this research; however, a possible inconvenience may be the amount of time you may be required to set aside to participate in the study.

8. How will my personal information be protected?

The researchers will keep all study records in a secure location. All electronic files containing identifiable information will be password protected. Any computer hosting such files will also have password protection to prevent access by unauthorized users. Only the members of the research staff will have access to the passwords. At the conclusion of this study, the researchers will publish their findings without disclosing any personally identifiable information.

Statement for **focus groups**:

“Please be advised that although the researchers will take every precaution to maintain confidentiality of information, the nature of focus groups prevents the researchers from guaranteeing confidentiality. We would like to remind participants to respect the privacy of fellow participants and not repeat what is said to others.”

9. Will I receive any payment for taking part in the study?

You will not receive any payment for taking part in this study. However, *icipi* will cover costs related to expenditures incurred as part of the study requirements e.g. sampling, recordkeeping and shipment of specimens.

10. What if i have questions?

If you have further questions about this project, you may contact

ProtusWanjala Mulati

Research Scientist

International Centre of Insect Physiology and Ecology

Duduvile Campus, Off Thika Road, Kasarani

P.O. Box 30772-00100, Nairobi, KENYA

Tel: +254 (20) 8632060, +254 (20) 8632000

Email: wanjaprot06@yahoo.com

11. Can I stop being in the study?

You do not have to be in this study if you do not want to. If you agree to be in the study, but later change your mind, you may drop out at any time. There are no penalties or consequences of any kind if you decide that you do not want to participate. You will be notified of all significant new findings during the course of the study that may affect your willingness to continue.

12. What if I am injured?

We do not expect that the study activities will cause you any physical injury.

13. Subject statement of voluntary consent

As I sign this form, I declare that I have understood its contents and agree to voluntarily enter this study. I have also had the opportunity to ask questions and received satisfactory answers. I understand that I can withdraw from the study at any time. A copy of this signed Informed Consent Form has been given to me.

[For **focus group** research, it may be useful to include a statement of non-disclosure that participants would agree to]

I agree to maintain the confidentiality of the information discussed by all participants and researchers during the focus group session. (If you cannot agree to the above stipulation please see the researcher(s) as you may be ineligible to participate in this study).

Name of Apiary (Print):

Participant's Name (Print):

Participant's Signature:

Date:

By signing below I indicate that the participant has read and, to the best of my knowledge, understands the details contained in this document and has been given a copy.

Name of Person

Signature of Person:

Date:

Obtaining Consent (Print):

Obtaining Consent (Print):

Appendix VI: Respondents filling Questionnaire in Gatundu



(Source: Field survey, 2015)

Appendix VII: Other Pesticide Residues detected in Pollen during the study period

Mean concentration levels at 9 apiary sites during LCMS/MS analysis ≥ 0.10 ppb was considered to be below limit of detection

Residue/ Apiary site	Lari	Gatundu	Ruiru	Thika	Thika IPM	Juja	Kikuyu	Karura Forest	Lenana /Ngong forest	Quality Standards
Concentration ($\mu\text{g}/\text{kg}$)										EU
Oxamyl	< LOD	< LOD	0.46	0.45	5.54	0.85	< LOD	< LOD	< LOD	50
Carbaryl	< LOD	< LOD	< LOD	< LOD			< LOD	< LOD	< LOD	10
Atrazine	< LOD	2.44	1.34	< LOD	16.07	1.36	< LOD	2.44	< LOD	50
Diazinon	< LOD	0.31	0.10	< LOD	4.12	3.99	< LOD	0.12	< LOD	
Metribuzin	< LOD	< LOD	0.32	3.82	0.37	< LOD	< LOD	< LOD	< LOD	
Aldicarb	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	
Dimethoate	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	
Diclorvos	< LOD	0.31	0.52	< LOD	0.73	0.48	< LOD	1.02	< LOD	
Cymoxanil	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	
Febuconazole	< LOD	0.17	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	
Pyraclostrobin	< LOD	0.37	0.41	0.34	0.53	0.71	< LOD	0.70	< LOD	
Fenazaquin	< LOD	1.44	1.68	1.68	2.10	2.55	< LOD	2.40	< LOD	
Hexaconazole	< LOD	1.56	< LOD	< LOD	0.18	0.10	< LOD	< LOD	< LOD	
Chlorophynipos-Methyl	< LOD	0.10	0.91	0.11	< LOD		< LOD	< LOD	< LOD	
Chlorophyniphos	< LOD	1.14	< LOD	< LOD	< LOD	3.15	< LOD	2.83	< LOD	
Chlorfeniphos	< LOD	< LOD	0.14	19.52	< LOD	< LOD	< LOD	< LOD	< LOD	
Carbendazim	< LOD	< LOD	0.26	< LOD	0.95	1.12	< LOD	< LOD	< LOD	
Propiconazole	< LOD	< LOD	0.58	0.10	0.18	< LOD	< LOD	< LOD	< LOD	
PrimiphosMethyl	< LOD	< LOD	0.16	0.11	0.63	< LOD	< LOD	0.14	< LOD	
Metalaxyl	< LOD	< LOD	< LOD	< LOD	0.67	< LOD	< LOD	< LOD	< LOD	
Bupirimate	< LOD	< LOD	< LOD	< LOD	0.47	< LOD	< LOD	< LOD	< LOD	
Clorp-Methyl	< LOD	< LOD	< LOD	< LOD	8.37	< LOD	< LOD	< LOD	< LOD	
Clofentezin	< LOD	< LOD	< LOD	< LOD	0.83	< LOD	< LOD	< LOD	< LOD	

