EFFECT OF PESTICIDES USE ON HONEYBEE (*Apis mellifera* L.) MORTALITY AND

HONEY PRODUCTION IN TRANSMARA WEST SUB-COUNTY, NAROK COUNTY,

KENYA

BY

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DECLARATION

Declaration by Student

This thesis is my original work and has not been submitted for any degree or any other academic award in any other university

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DEDICATION

This thesis is dedicated to my dear children; Laura, Michelle, Angela and Mercy who have been a source of happiness to me throughout this study.

ABSTRACT

The honeybee (*Apis mellifera L.*) produces honey and cross pollinates plants for improved socioeconomic wellbeing. However its colony populations globally and locally have been declining. In Transmara West Sub-county, hive colonization and honey yields have been low, which is due to the decline in honeybee population believed to be caused by pesticides use and pests attack. Although their relative contributions are unknown, Beekeepers suspect pesticides use hold a key role in colony population decline. This scenario has impeded optimal honey production. Previous studies in the study area focused on beekeeping suitability and potential and little on effect of pesticide use. The main objective of this study was to establish the effect of pesticides use on honeybee mortality and honey production. The specific objectives were: to analyze the effect of pesticide use on honeybee mortality and honey yield, examine pesticide residue levels in honeybee, honey and pollen and determine pesticide use patterns. The study adopted experimental and descriptive survey design. Sixteen apiaries were selected and two strong colonies in Langstroth hives identified per apiary and replicated thrice totaling to 94 colonies which acted as control and treatments. Traps were fixed at hive entrances and number of dead bees recorded at weekly intervals in March-October 2015. Pollen, honeybee and honey samples from the colonies were analyzed for Amitraz, Chlorfenvinphos, Cypermethrin, Deltamethrin and Malathion residues at SGS laboratories, using Queshers method. A population of 2500 beekeeping households was targeted and a sample of 330 respondents randomly drawn and administered with a questionnaire. Honeybee mortality rate and honey yields data among experimental sets were analyzed by one way ANOVA and mean separation using Turkey HSD test. Pesticides use data was analyzed using descriptive statistics. The results indicated that mortality rate in treated colonies (229±5.1) was significantly higher than in control colonies (73±11); MSD=4.6791, p=0.01. Honey yield in control colonies (16.0±1.0Kg) was significantly higher than in treated colonies (8.7±1.2Kg); (MSD=4.8425, p=0.024. Pests were controlled using pesticides (91%) mainly; pyrethroids (50%), formamidine (25 %) and organophosphorous (25%). Most farmers applied pesticides weekly (79%) during morning hours (93%) with 66% applying pesticides cocktails for efficacy purposes. About 83% disposed pesticides inappropriately. No residues were detected in all matrices thus honeybee products are safe for consumption. Pesticides use increased honeybee mortality rate hence reduced honey yields. Pesticides were handled haphazardly in the study area. Farmers should be sensitized on safe pesticides handling. This information will guide the development of proper pesticides handling strategies.

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LIST OF ACRONYMS AND ABBREVIATION

ANOVA – Analysis of Variance

AOAC – Association of Official Analytical Chemists

CCD – Colony Collapse Disorder

EU – European Union

GoK – Government of Kenya

HOAc - Acetic Acid

ICIPE – International Centre for Insect Physiology and Ecology

KALRO – Kenya Agricultural and Livestock Research Organization

Kg - Kilogram

KNBS - Kenya National Bureau of Statistics

KTBH – Kenya Top Bar Hive

LC-MS/MS- Tandem Liquid Chromatography/Mass Spectrometry/Mass Spectrometry

LD_{50} – Median Lethal Dose

LOD – Limit of Detection

LOQ – Limit of Quantification

MeCN – Acetonitrile

$MgSO_4$ – Magnesium Sulfate

mL - millilitre

MPH – Metres Per Hour

MRL – Maximum Residue Limit

NaOAc – Sodium Acetate

NBS – National Beekeeping Station

PAN – Pesticide Action Network

PCPB – Pest Control Products Board

PSA - Primary Secondary Amine

SGS – Societe General De Surveillance

SPE - Solid-Phase Extraction

SPSS – Statistical Package for Social Scientists

USA – United States of America

USD – United States Dollar

DEFINITION OF TERMS

Acaricide: A pesticide used to control external livestock parasites

Apiary: A place where bees are kept either for domestic or commercial purposes and ranges from a single hive to several hives

Chromatography: A technique for separation of mixture by passing it in solution or suspension through a medium in which components move at different rates

Colony: A group of honeybees comprising a queen, drones and worker bees living in a hive or swarming.

Forage: A wide search over an area by a bee in order to gather nectar, pollen and water

Tandem mass spectrometry: An analytical technique consisting of two mass spectrometers in series linked via a collision cell that is used for quantitative determination of molecules in a mixture upon separation by a chromatograph

Matrix: A material or component sampled for pesticide residue studies. In this study, honeybee, pollen and honey are the matrices for pesticides residue analysis

Mortality rate: It is the number of dead honeybees per station per week (The maximum threshold is 250 dead honeybees per station per week)

Pattern: a sequence discernible in the way in which something happens or is done. In this study, nature of pesticide, storage, time and frequency of use and disposal constituted use pattern.

Senescence: It is an age-specific decrease in physiological performance accompanied by an increase in mortality rate

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CHAPTER ONE

INTRODUCTION

1.1 Background to the study

The honeybee (*Apis mellifera L.*) is very important in production of honey, pollen, propolis, wax, royal jelly and bee venom besides cross pollination of plants (Klein *et al*., 2007 and Gallai *et al*., 2009). The global honey production stands at 1.4 million tons annually with China producing 20 %, while Turkey, Argentina and USA each producing 6 % of global honey. The rest is produced from other regions around the globe (FAO, 2010). In Africa; Ethiopia, Tanzania and Kenya are the leading honey producers with 41,233, 28,678 and 25,000 tons annually in that order (Wainwright, 2005). Crops pollinated by honeybees have greater returns (Kasina *et al*., 2010). An economic evaluation of the pollination service provided by bees on the main agricultural food crops was about USD 208 billion i.e. 9.5% of the total value of the global food production (Mutuku *et al*., 2013). However, the realization of this potential is being impeded by constraints such as; climate change, inappropriate pesticides uses, pests and diseases that act synergistically (Sanford and Jamie, 2011). Pesticides use is suspected by Scientists and beekeepers to hold a key role in honeybee colony population decline (Henry *et al*., 2012). This was because they induce behavioral changes that result in high honeybee mortality, honey and pollen contamination (PAN, 2012; Mutuku *et al*., 2013).

Global honeybee populations have been declining with North America and European beekeepers routinely reporting a 30 % loss of their managed colony populations over the last 30 years (VanEngelsdorp and Meixner, 2010). However no single factor has strongly been linked to colony losses (Alaux *et al*., 2010). It is believed that several factors act synergistically to reduce

colony survival, with pesticides playing a key role in colony decline (Vidau *et al*., 2011). This was because pesticides impair bee homing ability, learning and memory, reduced foraging, travel and olfactory distortion (PAN, 2012 and Whitehorn *et al*., 2012). For instance up to 32 % of honeybees exposed to sub-lethal levels of pesticides in France failed to return to the hive, effectively doubling the natural loss rate of foraging workers (Henry *et al*., 2012). Furthermore, beekeepers near flower farms and tea estates in Uganda and Kenya have complained of decline in honeybee colony populations and attributed it to pesticides poisoning (Kajobe *et al*., 2009). Thus although pesticides exposure even at sub-lethal doses impact negatively on honeybees, and given its important role in crop production through cross pollination, no study has been done to understand the role of pesticides use on honeybee mortality rate in the sub-county.

In Kenya honey production has been declining with the national average annual yields in 2005, 2006 and 2007 being 20.28 kg, 15 kg and 9.3 kg/colony in that order (NBS, 2007). In Transmara West Sub-County, the average 2009 honey yield for a langstroth hive was 13.2 Kg compared to 18 Kg in the past (Honey Care Africa, 2010). This yield decline was attributed to pesticides use and habitat modification (Carroll, 2002; Mutungi *et al*., 2003; MacOsore *et al*., 2005). While these studies illustrate important highlights on effect of pesticides use on honeybee mortality rate and yields. The studies nevertheless could not consistently link a single factor to colonies decline. Therefore they concluded that the factors act synergistically with pesticides playing a key role in the decline. However despite continuous pesticides use in the study area no empirical information was available that links use and effects of pesticides on honeybee mortality rate and yields. Therefore this study determined the contribution of pesticides use on honeybee mortality rates and honey yields, components that have been missing in past studies in the region.

Contamination of honeybee products by pesticides is widespread, for example, over 129 different pesticide residues were detected in 90% of honeybee colonies in the USA (Mullin *et al*., 2010). Further organochlorine pesticides were found in most Portuguese and Spanish honey samples (Cristina *et al*., 2003) while organohalogens and organophosphorous residues were detected in Brazilian honey (Sandra *et al*., 2007). In Switzerland, no pesticide residues were detected in 27 honey samples (Bogdanov *et al*., 2003). Furthermore only four pesticides residues mostly at low levels were detected while screening honey samples from 24 apiaries across Kenya (Muli *et al*., 2014). Analysis of honey samples collected from across 13 beekeeping zones in Kenya detected no residues (Orina, 2012). The high pesticides residues incidences in some areas were attributed to high pesticides application rates (Reus and Leendertse, 2000).

Due to safety concerns arising from inappropriate pesticides use that generate considerable amount of residues often higher than their MRL, the Codex Alimentarius established MRLs for pesticides. The MRL for Cyhalothrin, is 0.01 mg/Kg while Amitraz, Cypermethrin, Deltamethrin was 0.005 mg/Kg (FAO/WHO, 2010). Whereas these studies provide important insights on the extent of pesticides contamination on honeybee products, they reported mixed results. Pesticides residues were detected in some products in some areas while in others very low or no pesticides residues were detected. However despite pesticides use in Transmara West Sub-County and given that MRL is a key measure of quality and safety, honey and pollen pesticides residue information was notably missing. Therefore this study screened honey, pollen and honeybee matrices for pesticides residues to assure product quality and safety for the markets. This will help boost consumer confidence of the regions honey raising the residents livelihood.

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About 4.6 million tons of chemical pesticides, worth USD 40.5 billion are annually applied to the environment globally with Europe being the largest consumer. Asia is second while Africa accounts for only 4 % of this volume (WenJun *et al*., 2011). China, USA, France, Brazil and Japan economies are the leading pesticide consumers globally accounting for 1.5, 0.4, 0.12, 0.12, and 0.065 million tons in that order (FAO/WHO, 2010; WenJun *et al*., 2011). South Africa consumes 0.10 million tons accounting for half of Africa's pesticides consumption (FAO, 2010). Sadly only one percent of sprayed pesticides effectively hit their targets while 99 % are released to non-target environment and finally absorbed by almost every organism (FAO, 2010) causing extensive damage to biodiversity. Further the annual average pesticides application rate in Latin America is 7.17 kg a.i./ha compared to 3.12 kg a.i./ha for Asia and 1.23 kg a.i./ha for Africa (WenJun *et al*., 2011). Thus it can be concluded that since pesticides use in Africa's agriculture was low, their risks and impacts must also be correspondingly lower (Ebenebe *et al*., 2001; Waichman *et al*., 2007). However this depends on ecosystem tolerance and hazards arising from inappropriate storage and applications (Waichman *et al*., 2007). Use of extremely harmful pesticides even at low rates is quite detrimental to the environment (Macharia *et al*., 2009). This is compounded by poor disposal of pesticides, containers and extent of use (Otieno *et al*., 2010; Mutuku *et al*., 2013). Many developing countries including Kenya have adopted pesticides use without farmer education and with limited extension services. Thus many pesticides are often used injudiciously without clear direction hence impacting negatively on non-target organisms such as honeybees; hence cross pollination. This in turn lowers crop yields threatening livelihoods. This observation was equally supported by that of Kolankaya *et al*., (2002).

Studies indicate that Transmara West Sub-County is an agro-pastoral area with the main pest control method being pesticides (Magembe *et al*., 2014). Further it has a varied edaphic and climatic conditions ideal for a range of plant vegetation with nectar and pollen for sustaining a large number of honeybee colonies (Kiyiapi, 2000; Ogweno *et al*., 2009). These studies have illustrated important disparities regarding pesticides handling. While they indicate significantly higher use intensities in developed countries, in developing countries their effects are highly negative on bee colonies due to extremely harmful pesticides used here. However save for pesticides classes used and beekeeping potential, empirical information on pesticides use patterns was missing in the study area. Thus this study determined the pesticide use patterns whose findings will help in guiding pesticides handling policy formulation in the sub-county and beyond.

1.2 Problem statement

Despite the varied edaphic and climatic conditions in Transmara region supporting a range of plant vegetation, that provide nectar and pollen making it suitable to sustain a large number of bee colonies, hive colonization and honey yields have been low. Honeybee colony populations have been declining probably due to haphazard pesticides use by farmers. Although pesticides use increase crop and animal productivity by controlling harmful insects, they inadvertently threaten the honeybee by inducing behavioral malfunctions that jeopardize colony survival. Further, they compromise the quality and safety of honeybee products. Nevertheless information on their effects on the honeybees` mortality rate and ability to pollinate plants and gather nectar is scanty. Likewise, analysis of honeybee and its products for pesticide residues in the area has not been done despite their potential detrimental risks on human health. In addition pesticide use intensity, timing, frequency and disposal in the area have not been documented. It was thus

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imperative to establish pesticide use patterns among farmers and pesticide effects on the honeybees` colonies population, honey and pollen production in Transmara West Sub-County in order to help address current challenges such as biodiversity loss, food insecurity and malnutrition in the region considering the ecological role of honeybee.

1.3 Objectives of the study

The main objective of this study was to establish the pesticides use patterns and their effect on honeybee mortality rate and honey production in Transmara West Sub-County.

The specific objectives were;

1. To analyze the effect of pesticides use on honeybee mortality rate and honey yields in Transmara West Sub-County

2. To examine pesticide residue levels in honeybee, honey and pollen in Transmara West Sub-County

3. To determine pesticide use patterns among farmers in Transmara West Sub-County

1.4 Research hypotheses

1. H_0: Pesticides use has no significant effect on honeybee mortality rate and honey yields in Transmara West Sub-County

2. H_0: There are no significant pesticides residues in honeybee, honey and pollen in Transmara West Sub-County

3. H_0: There are no discernible patterns of pesticides use among farmers in Transmara West Sub-County

1.5 Justification of the study

The increased use of pesticides in agriculture has raised a number of ecological concerns such as poisoning of non-target organisms (Kevan, 1999). Hence pesticides use patterns assessment needs to be conducted to develop strategies that effectively control pests while safeguarding honeybees and maintaining environmental integrity (Chan *et al.,* 2006). Since Chlorfenvinphos, (Amitraz, Deltamethrin, Malathion and Cypermethrin) pesticides belong to toxicity classes Ib and II respectively (WHO, 2010), monitoring honeybee mortality rate, is very important to understand their potential honeybee poisoning risks. Due to its wide area of patrol and intense foraging activity, the honeybee can also be used as a bio-indicator to determine the degree of environmental contamination due to pesticides (Porrini *et al*., 2003). In addition, detection of pesticide residues in honeybee products is a serious health concern among consumers (Karazafiris *et al.,* 2011). Further, the growing demand for organic honey in markets such as EU, though with stringent export regulatory requirements demands that products must be screened to guide farmers, other stakeholders on pesticides handling in farms to meet and maintain export compliance and consumer safety. The findings of this study will help improve farmers' agricultural practices and also in the objective policies formulation for apiculture subsector.

1.6 Scope of the study

This study was conducted in Lolgorian, Angata, and Kilgoris Divisions of Transmara West Sub-County, Narok County, Kenya in 2015; March – July (long rains) and August – November (short rain) seasons. It determined the effect of pesticides use on honeybee mortality rate and honey yields and measured the pesticides residues in honeybee, honey and pollen. In addition it focused on pesticide use patterns among beekeepers and their effect on honeybees' survival.

CHAPTER TWO

LITERARURE REVIEW

2.1 Introduction

This chapter outlines the global pesticides consumption, routes of pesticides exposure to honeybee and hive products, formulations and mode of action of pesticides. Further it highlights the contribution of pesticides to honeybee mortality rates. The section also reviews information on pesticide residue dynamics in honey, honeybee and pollen; residue limits and the analytical techniques used in the residues determination. In addition, conceptual framework indicating the relationship among the variables in the study is described.

2.2 Effect of pesticides use on honeybee mortality and honey production

2.2.1 Classes and formulations of pesticides

Pesticides are classified into groups based on various criteria such as; their chemical structure i.e. organophosphates, pyrethroids, organochlorines, carbamates, neonicotinoids etc., mode of action; systemic or contact, target organism; insecticides, acaricides, herbicides, fungicides, bactericides, nematicides and synthesis whether synthetic or natural (Emmanouel *et al*., 2011). Most fungicides, herbicides and miticides are unstable and disintegrate quickly after use hence relatively non-toxic to honeybees (Bogdanov, 2006). However synthetic pyrethroids are highly toxic to honeybees and cannot be applied to blooming crops when bees are present without causing serious injury to the colonies (Bogdanov, 2006). Dust formulations are typically hazardous than sprays because they are picked up on honeybee hairs (Kolankaya *et al*., 2002). However, wettable powder would remain toxic in the field for longer periods than emulsifiable concentrates (Magic, Keshet, Sypertix and Steladone), while granular insecticides are less

hazardous to honeybees (Kolankaya *et al*., 2002). Drift of spray application can cause significant problems when it reaches the colonies or adjacent flowering crops or weeds. It is therefore advisable to locate apiaries far from spray race or pesticides be applied when the wind speed is below 10 mph (Garcia *et al*., 1996). Further pesticides that degrade within a short time are usually applied without much risk when honeybees are foraging (Wallner, 2003).

2.2.2 Exposure of honeybee and hive products to pesticides

Sandford and Jamie (2011) observed that while foraging, field bees may range as far as two to five miles from a colony. Gregorc and Ellis (2011) concluded that about 10,000 - 25,000 honeybee workers of a colony make an average of ten journeys every day to explore roughly 7 Km^2 in the area near their hive while gathering nectar and pollen from flowers. They usually forage systematically, not randomly, and once a food source is found, bees prefer to work that particular source to exhaustion before changing plants (Chauzat *et al*., 2009). This kind of resource partitioning by honeybee colonies accounts for the inconsistency observed many times between colonies undergoing pesticide poisoning in the same location (Marten, 2004). The bees are not all working the same plants and so some are affected more than others. Often it is those bees with established flight patterns located in an area before a pesticide is applied that are most affected (Sandford and Jamie 2011).

During foraging process, various microorganisms, chemical products, and particles suspended in the air from industrial, agricultural and domestic activities are intercepted by these workers and retained in the hair of their body surfaces, or inhaled and attached to their trachea (Devillers and Pham-Delegue, 2002a). In many cases, these chemicals are pesticides which encompass an array

of compounds designed to repel or kill insects (insecticides), plants (herbicides), fungi (fungicides) and other organisms considered pests (Grecorc and Ellis, 2011). Though honeybees are non-target organisms for most pesticide applications, they nevertheless get exposed to pesticides while foraging, drinking water from rivers, lakes and ponds, breathing, and during flight (Maya *et al*., 2012). These pesticides may be brought inadvertently to the colony where their levels are concentrated further in the waxy nest infrastructure and consequently negatively affecting the colony. This denies the environment the crucial honeybee pollination service that in the long run impacts the residents livelihood adversely (Ellis, 2010; Weick *et al*., 2002).

2.2.3 Mode of action of pesticides

Pyrethroids, in general, interfere with normal production and conduction of nerve signals in the nervous system. It acts on nerve membranes by delaying the closing of the activation gate for the sodium ion channel (Tomlin, 2006) hence killing target pests by blocking the voltage-gated sodium and calcium channels (Davies *et al*., 2009). Deltamethrin is effective against insects via ingestion and direct contact; it expresses a strong knock-down effect while Amitraz, a non-systemic insecticide and acaricide, causes stimulation of neuronal activity killing the target (Tomlin, 2006). Cyhalothrin penetrates the insect cuticle, disrupting nerve conduction within minutes; this leads to cessation of feeding, loss of muscular control, paralysis, and eventual death (Kaijun 2012). Cypermethrin inhibits the γ-aminobutyric acid receptor, causing excitation and convulsions, inhibits calcium uptake by nerves and inhibits monoamine oxidase, an enzyme that breaks down neurotransmitters (Anand *et al*., 2012). Chlorfenvinphos, an organophosphate pesticide acts by inhibiting acetylcholine esterase (Tomlin, 2006).

Malathion is toxic via skin contact, ingestion, and inhalation exposure. They bind to the enzyme acetylcholinesterase (AChE) at nerve endings throughout the bodies of insects and other organisms resulting in overstimulation of the nervous system leading to eventual death of insects (Journal of Pesticide Reform, winter 2003). Neonicotinoids are acetylcholine mimics and act as nicotinic acetychloline receptor agonists. They cause persistent activation of cholinergic receptors which leads to hyper excitation and death (Jeschke and Nauen, 2008). The Phenylpyrazoles, including Fipronil, bind to "-amino butyric acid (GABA)-gated chloride ion channels and block their activation by endogenous GABA, leading to hyper excitation and death of the pests (Gunasekara *et al*., 2007). Many of the pesticides to which honeybees are exposed have insecticidal properties and may be harmful to bees. For example, pesticides are known to lower the developmental rate of queen honeybee, increase the occurrence of queen rejection, and lower queen weight (Nasr and Wallner, 2003; Pettis *et al*, 2004). In addition, they cause honeybee cardio toxicity (Papaefthimiou and Theophilidis, 2001), and affect forager bee mobility and communicative capacity (Medrzycki *et al*., 2003).

Honeybees have been reported to be susceptible to many pesticides more than other insects (Henry *et al*., 2012). Pesticides impact on their immune systems, predisposing them to diseases and interfering with brood development and shorten lifespan of adult honeybees (; Pettis *et al*., 2012; Wu *et al*., 2012 and Desneux *et al*., 2007). The recent sequencing of the honeybee genome found that relative to other insect genomes, the honeybee genome is markedly deficient in the number of genes encoding detoxification enzymes, including cytochrome P450, monooxygenases (P450s), glutathione-S-transferases, and carboxylesterases (Claudianos *et al*.,

2006). This relative lack of detoxicative genes in the honeybee genome reduces the chances of gene response following pesticides exposure (Claudianos *et al*., 2006).

2.2.4 Effect of pesticides use on honeybee mortality rate

Naturally, honeybees like other living organisms exhibit senescent decline. Senescence is defined as an age-specific decrease in physiological performance accompanied by an increase in mortality rate (Dukas, 2008b). However in the recent past, honeybees have been dying off at unprecedented rates around the world (PAN, 2012), hence generating interest among scientists. Oldroyd (2007) concluded that colony collapse disorder (CCD) is a recent, pervasive syndrome affecting honeybee (*Apis mellifera L*) colonies in the Northern hemisphere, and is characterized by a sudden disappearance of honeybees from the hive. North America and European beekeepers have routinely reported up to 30 % losses of their managed colony populations over the last 30 years (VanEngelsdorp and Meixner, 2010). Multiple causes of CCD have been proposed, such as pesticides use, pathogens, parasites, and natural habitat degradation (Cox-Foster *et al*., 2007; Naug, 2009). However, the relative contribution of those stressors in CCD events remains unknown (Henry *et al.*, 2012) since no study has strongly linked a single factor to colony losses (Alaux *et al*., 2010). Thus the belief that these factors act synergistically to weaken colonies, with pesticides playing a key role in colony decline (Vidau *et al*., 2011). This was because pesticides induce honeybee malfunction in navigation and homing ability, impaired memory and reduced foraging and olfactory distortion (PAN, 2012 and Whitehorn *et al*., 2012). Although no single pesticide has been found to cause CCD, the synergistic effects of multiple pesticide exposures may be contributing to the decline in colony population (Johnson *et al*., 2010).

Honeybees are extremely sensitive to pesticides; the number of dead bees in front of the hive is therefore the most important variable to be considered for these contaminants (Porrini *et al*., 2002). This varies according to a number of factors such as the toxicity of active ingredients used (LD_{50}), the presence of honeybees on the sites at the time of chemical treatment, the means used to distribute the pesticide and the presence of wind (Porrini *et al*., 2003). For instance up to 32 % of honeybees exposed to sub-lethal levels of pesticides in France failed to return to the hive, effectively doubling the natural loss rate of foraging workers (Henry *et al*., 2012). In addition beekeepers near flower farms and tea estates in Uganda and Kenya have complained of decline in honeybee colony populations and attributed it to pesticides poisoning (Kajobe *et al*., 2009). Thus although pesticides even at sub-lethal doses impact negatively on honeybees, and given that farmers suspect as such, no study has been done to understand the impact of pesticides use on honeybee mortality rate in the Sub-County.

Many honeybees directly struck by pesticides will not have enough strength to return to their hive and will die in the field or during their return flight (Porrini *et al*., 2002). Others only marginally hit while visiting the flowers of the treated species or gathering nectar and pollen from spontaneous species contaminated by drift will eventually die in the hive hence acting as a direct indicator (Sanford and Jamie, 2011). In the case of compounds that are not particularly dangerous, the insect acts as an indirect indicator providing information in form of residues (Celli *et al*., 1996). This monitoring scheme will yield results such as weekly mortality, active ingredients responsible for bees kill, periods and areas at highest risks (Porrini *et al*., 2003). In the event that mortality rate exceeds the critical threshold of two hundred and fifty (250) dead honeybees per week per station, laboratory analyses are carried out (Porrini *et al*., 2002).

Most pesticides programs for monitoring honeybee mortality rates have been oriented to the determination of the impacts of acaricides that are apiculture based (Walner, 1999; Menkissogl *et al.*, 2001). Further, most pesticides regulatory authorities globally focus mainly on lethal concentrations yet there is evidence that sub-lethal pesticides doses cause alterations in honeybee's physiological functions (Henry *et al*., 2012; PAN, 2012; Whitehorn *et al*., 2012). However attention has since shifted to studies on pesticides used for crop and livestock protection and introduced into the hive by contaminated honeybees (Al-Rifai and Akeel, 1997). These studies have indeed illustrated the various constrains that impact on honeybees performance. Nevertheless the studies could not consistently link a single factor to honeybee colonies decline. Therefore they concluded that these various stressors acted synergistically with pesticides playing a key role in the decline. However despite pesticides use in the study area, no empirical information was available that links use and impacts of pesticides on honeybee mortality rate. The determination of pesticides use effect on honeybee mortality will help in conservation of biodiversity and maintenance of cross-pollination, a vital ecosystem service.

2.2.5 Effect of pesticides use on honey yields

Honey production globally and in Kenya, has been declining. The Kenyan yield in 2005 was 25,000 tons (Wainwright, 2005) against a potential of 100,000 tons (GoK, 2008). The average annual honey yields in Kenya in 2005, 2006 and 2007 were 20.28, 15 and 9.3 kg/colony in that order with Transmara West Sub-County having an annual honey yield of 18 Kg/colony (Carroll, 2002; NBS, 2007). An evaluation of log hives and KTBH for honey yields over a two year period in Cheptuya area found average annual yields of 18 Kg and 47 Kg of honey respectively

(MacOsore *et al*., 2005). This KTBH yield was comparable to the Rwandan Langstroth honey yield of 48 Kg (Nienke and Zunderdorp, 2008). This was due to the difficulty in attracting bees to Langstroth hives (Honey Care Africa, 2010). Further, pesticides use reduces worker bees and bee forage respectively hence low yields. Despite this scenario, no information linking pesticides use and honey yield was available in the sub-county, components this study determined.

2.3 Pesticide residue dynamics in honey, honeybee and pollen

Pesticides, especially herbicides have been found to contaminate honeybees and pollen more than honey (Celli *et al*., 1996). Studies conducted on North American honeybee colonies in 2007 and 2008 found 121 different pesticides and metabolite residues in wax, pollen and honeybees' samples but traces in honey samples (Mullin *et al*., 2010). Further organochlorine pesticides were found in most Portuguese and Spanish honey samples (Cristina *et al*., 2003) while organohalogens and organophosphorous residues were detected in Brazilian honey (Sandra *et al*., 2007). However in Switzerland, no pesticide residues were detected in 27 honey samples analyzed for pesticides residues (Bogdanov *et a*l., 2003).

In Kenya, efforts have been made to examine pesticides residues in various matrices suspected of pesticides exposure. Otieno *et al*., (2010) determined the concentrations of carbofuran residues in water and soil samples from agricultural farmlands in Isiolo and Laikipia Districts, Kenya. He found high concentrations of carbofuran demonstrating extensive Furadan use in the two areas posing risks to man, domestic and wild animals drinking the water. Wandiga (2001) studied the distribution of organochlorine pesticides along the Indian Ocean coast of Kenya and found that the lowest concentration of pesticides was found in water followed by sediment and fish.

However, some attempts made to determine pesticides residues in honeybee products in Kenya obtained mixed results. Orina (2012) evaluated the levels of pesticides residues and found none in honey samples on sale in Mwingi, Kitui, Ntubo, Tharaka, Embu, Mbeere, Timboroa, Turbo, Malaba forest, Lenana forest, Thika Kakuzi, Kakamega forest and Taita Taveta beekeeping zones of Kenya. Further, Muli *et al*., (2014) performed pesticide analysis on honey and pollen samples from 13 sites across Kenya. Only four pesticides; 1-naphthol, chlorothalonil, chlorpyrifos and fluvalinate out of 171 were found to be present mostly at very low levels (< 0.05 Mg/Kg). The absence or relatively low pesticides concentration in honey compared to other matrices may be attributed to a filtering effect of honeybees. Studies have established that honeybees indeed decrease initially high pesticide nectar concentrations so that the final concentration in honey was much lower, mostly by a factor of about 1000 (Schur and Wallner, 2000). However Bonmatin *et al*., (2005) and Kievits (2007) made a contrary finding; they concluded that any pesticide in the nectar was concentrated at least four times in honey, which is stored for later use.

2.3.1 Pesticides residue limits

Pesticide residues in honey and hive products are a sensitive topic as honey and bee products (bee pollen, royal jelly, beeswax and propolis) are perceived as pure and natural food (Heinkelein, 2011). There are maximum residue levels (MRLs) for pesticide residues in honey, royal jelly and bee pollen given in regulation 396 of the year 2005, (EC) 470/2009 and (EU) /37/2020 (WHO/FAO, 2010). According to article 18 of this regulation a default MRL of 0.01 mg/kg was set for those products for which no specific MRL is set out in Annexes II or III, or for

active substances not listed in Annex IV. For example, the Acceptable Daily Intake (ADI) for Deltamethrin is 0.01 mg/kg (Tomlin, 2006). The MRL for Cyhalothrin was 0.5 as established by Codex Alimentarius (FAO/WHO, 2010). In pollen and honey, the LOQ for, Amitraz, Cypermethrin, Deltamethrin is 0.01mg/Kg while that for Chlorfenvinphos, Cyhalothrin Malathion and Diazinon is 0.005mg/Kg (FAO/WHO 2010).

Inappropriate pesticides use generates residues often higher than MRL. Given that MRL is a measure of product quality and safety (Heinkelein, 2011) and that pesticides are used in the study area (GoK, 2008), there was no pesticides residue information available. Although these studies provided valuable information regarding environmental contamination by pesticides, they obtained mixed results. Some studies detected pesticides residues while others did not. Further some matrices recorded higher residues than others. Besides no pesticides residue information yet considerable amounts of pesticides are consumed in the Sub-County. Therefore the study screened honey, pollen and honeybee matrices for pesticides to assure the market of product quality and safety. This will ensure good health while attracting higher premium market resulting in improved socioeconomic wellbeing of the residents of the study area.

2.3.2 Analysis of pesticides residues

Contaminations of honeybees, pollen and honey by pesticides have been monitored using various schemes. Honeybees more than its products, have been used as biological monitors for pesticide contamination of geographic regions (Celli *et al*., 1996). In these monitoring schemes, pesticide residues have been determined using chromatographic techniques. Gas chromatography (GC) is still the method of first choice for the analysis of pyrethroid residues with various detectors such

as GC with electron capture detector (GC-ECD) (Sandra et al., 2007; Su *et al*., 2007; De Pinho *et al*., 2010), GC-mass spectrometry (GC-MS) (Albero *et al*., 2004; Beltran *et al*. 2003; Kazuaki *et al*. 1997; Tagami *et al*. 2009), high performance liquid chromatography-ultraviolet (HPLC-UV) (Metwally *et al*. 1997), and HPLC-mass spectrometry(HPLC-MS) (Klein and Alder, 2003).

However, since honey or pollen contaminated at ppm or ppb levels with pesticides are known to impair honeybee health (Halm *et al*., 2006; Desneux *et al*.,2007, Johnson *et al*., 2009), it is important to use sensitive analytical technologies. One such technology is the recently developed liquid chromatography-tandem mass spectrometry (LC/MS-MS) QuEChERS method (Bonmatin *et al*., 2005; Chauzat *et al*., 2006). The QuEChERS method has since been modified to the most current AOAC Official Method 2007.01. It is quick, easy, cheap, effective, rugged and safe multiresidue analytical method (AOAC, 2007).

2.4 The pesticides use patterns

Adequate pesticides use ensures higher productivity gains in agriculture to meet the global demand for food security although their negative environmental impacts cannot be ignored either (Hamilton or Crossley, 2004). About 4.6 million tons of chemical pesticides worth USD 40.5 billion are annually applied to the environment globally with Europe being the largest consumer, followed by Asia with Africa accounting for only 4 % of this volume (WenJun *et al*., 2011). China, USA, France, Brazil and Japan economies are the leading pesticide consumers globally accounting for 1.5, 0.4, 0.12, 0.12, and 0.065 million tons in that order (FAO, 2010; WenJun *et al.,* 2011). South Africa consumes 0.10 million tons accounting for half of Africa's pesticides consumption (FAO, 2010). Sadly only 1% of sprayed pesticides effectively hit their targets while

99% are released to non-target environment and finally absorbed by almost every organism (FAO, 2010). South Africa's registered pesticides products is about 3000 (Dabrowski, 2015), three times Kenya's 1100 pesticide formulations registered with annual use of 8,000 metric tons of pesticides (PCPB, 2013), owing to its agriculture based economy (Birech and Benhard, 2006).

The average pesticide application rates differ considerably across regions. For example the Latin America and Asia rates are 7.17 kg a.i./ha and 3.12 - kg a.i./ha respectively compared to Africa's 1.23 kg a.i./ha, (Repetto and Baliga, 1996). Further most agricultural activities in Africa are small-scale farming systems, viewed as low input, with low use of pesticides (Ebenebe *et al*., 2001). Since the volume of pesticides used in Africa is much lower than elsewhere, the risks and impacts may be correspondingly lower (Ebenebe *et al*., 2001; Waichman *et al*., 2007). However this would ignore hazards arising from the use of toxic pesticides, poor handling practices and inadequate pesticides regulation (Waichman *et al*., 2007; Gitonga *et al*., 2010). For instance 50% of all pesticide related illness and 72.5% of reported fatal pesticide poisonings occur in developing countries yet they account for only 25% of global pesticides used (Harris, 1999).

It is expected that farmers follow dilution instructions labeled on the pesticide container and application done when honeybees are not working, preferably in the early evenings (Sandford and Jamie, 2011). However due to low literacy levels, measurements are rarely adhered to, resulting in either higher or lower pesticide concentrations (PCPB, 2005). The use of low concentrations results in resistance to pesticides by the target organisms causing economic losses (Marten, 2004). Conversely, the use of high pesticides concentrations may poison and kill non-target and beneficial organisms such as honeybees whenever they fly through a cloud of

pesticide dust or spray or walk on treated parts of plants (Bogdanov, 2006). The spraying of pesticides to livestock and crop fields during the day especially morning hours exposes honeybees to pesticides since this is the time when foragers are most active in the field gathering nectar, pollen and water (Sandford and Jamie, 2011).

The industrialized countries have developed sound pesticides waste disposal and management systems. For example triple rinsing of empty pesticides containers transforms them from hazardous to non-hazardous status. This coupled with obsolete pesticides collection schemes has made waste disposal very effective in many European and other developed countries (FAO, 2008). In addition, they have a robust infrastructure for disposal of obsolete pesticides such as incinerators. This is however not the case in the third world countries as most have no disposal infrastructure let alone disposal policies (FAO, 2008). This was despite the quantities of obsolete pesticides in Africa alone being more than 20,000 tons, which will cost up to US$150 million to destroy (Harris 1999). In addition, most African farmers have not abandoned crude pesticides disposal methods such burying or throwing containers away or pouring excess diluted pesticides (Gitonga *et al*., 2010). Thus these factors evidently predispose the physical and biological environment including honeybees to hazardous pesticides, hence the necessity of the study.

In Kenya, several studies have been carried out to understand pesticides use patterns and application regimes. Nyakundi *et al*. (2010) observed that pesticides were readily available and widely used in horticultural farms in Central and Rift valley provinces contaminating water bodies resulting in death of fish in nearby rivers. Further Mutuku *et al*., (2013) observed that majority of tomato farmers in Kathiani exposed themselves to pesticides hazards during

handling. Macharia *et al*., (2009) concluded that the vegetable sub-sector potentially has environmental pesticide negative impacts. Nderitu *et al*., (2007) found that Kenyan farmers applied insecticides up to 15 times during a single cropping season for crops like French beans. Transmara West Sub-County is an agro-pastoral area with the main pest control method being pesticides (Pyrethroids; Deltamethrin, Cypermethrin and organophosphorous (Steladone, Diazinon, Malathion and Amitraz) (GoK, 2008; Magembe *et al*., 2014). The sub-county has varied edaphic and climatic conditions ideal for a range of plant species with nectar and pollen for sustaining a large number of honeybee colonies (Kiyiapi, 2000; Ogweno *et al*., 2009).

These studies have provided important information on pesticides accessibility, use patterns and their potential risks to man and the environment. Further they highlighted the beekeeping potential of the study area. However although the effect of pesticides use on honeybees' has been documented in the developed economies, this component was notably missing in the study area. Besides pesticides use patterns studies have not been carried out in the sub-county and how they impact beekeeping and production despite being the main agricultural pests control method. Therefore this study determined the pesticide use patterns whose findings will help in guiding the sub-county and national pesticides handling policy formulation.

2.5 Conceptual framework

Pesticides remain indispensable in increasing crop and livestock production to satisfy the global demand for quality and adequate food supply. However they comprise an array of compounds that are designed to repel or kill pests. Unfortunately, the honeybee has been one of the beneficial species that is threatened despite being a non-target in most pesticides applications.

Exposure and effects of pesticides use on honeybees are influenced by factors such as; time of application, distance from colony to exposure point, physiological stage of forage plants, dosage and nature of pesticides. Ideally appropriate pesticides application away from honeybee colonies, at the right time and dosages as prescribed minimizes exposure and the effects they would have on honeybees. However exposure to high pesticide doses result in outright bees kill while exposure to sub lethal doses induce behavioral impairment such as reduced foraging, homing and navigational malfunction and reduced queen production. Consequently honeybee mortality rate may increase. In addition rate of hive colonization and honey production may reduce depending on how these factors interact.

Independent variable   Intervening variable   Dependent variable
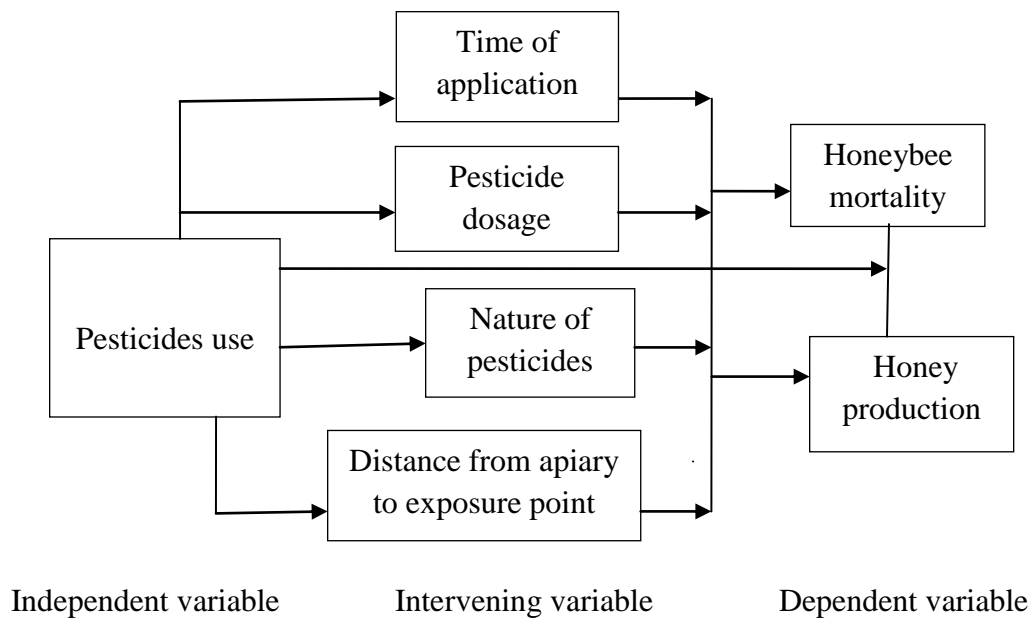
Figure 2.1. Relationship among pesticides use, honeybee mortality and production (Author, 2015)

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CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter describes the study area and outlines the designs used to carry out the study including collection of data. It describes the population and sample sizes, sampling strategy and instruments of data collection. It further details the collection of samples, preservation and analytical techniques used as well as data analysis and presentation of information.

3.2 Description of the study area

Transmara West Sub-County is located in Narok County, Kenya and consists of four administrative divisions namely: Lolgorian, Angata, Kilgoris and Keiyan. It covers an area of approximately 2900 km^2 with Maasai Mara game reserve occupying 312 km^2. The Sub-county lies between latitudes 00 50` S and 10 50` N and longitudes 340 35`E and 350 14` W. It is divided into highlands (between 2200 m and 2500 m above sea level) and the plateau (1500 m to 2200 m above sea level). It borders the Republic of Tanzania to the South, Migori County to the West, Kisii, Nyamira and Bomet Counties to the North. The dominant elevations are between 1800m to 1950m interrupted by rocky eroded hills. Annual temperature ranges from 14.8 ^{0}C to 20.3 ^{0}C. The sub-county receives a bimodal rainfall which in normal years is well distributed throughout the year with peaks in April (long rains) and December (short rain) seasons.

The sub-county is suitable for livestock production and as well arable agriculture with current dominant activities being beef livestock rearing and maize farming. Other enterprises include mining, sand harvesting, beekeeping, dairy farming and cash cropping such as sugar cane and

tea. Besides these, another important income generating resource for the Sub-County is the Maasai Mara Game Reserve, where the Narok County government obtains a lot of revenue.
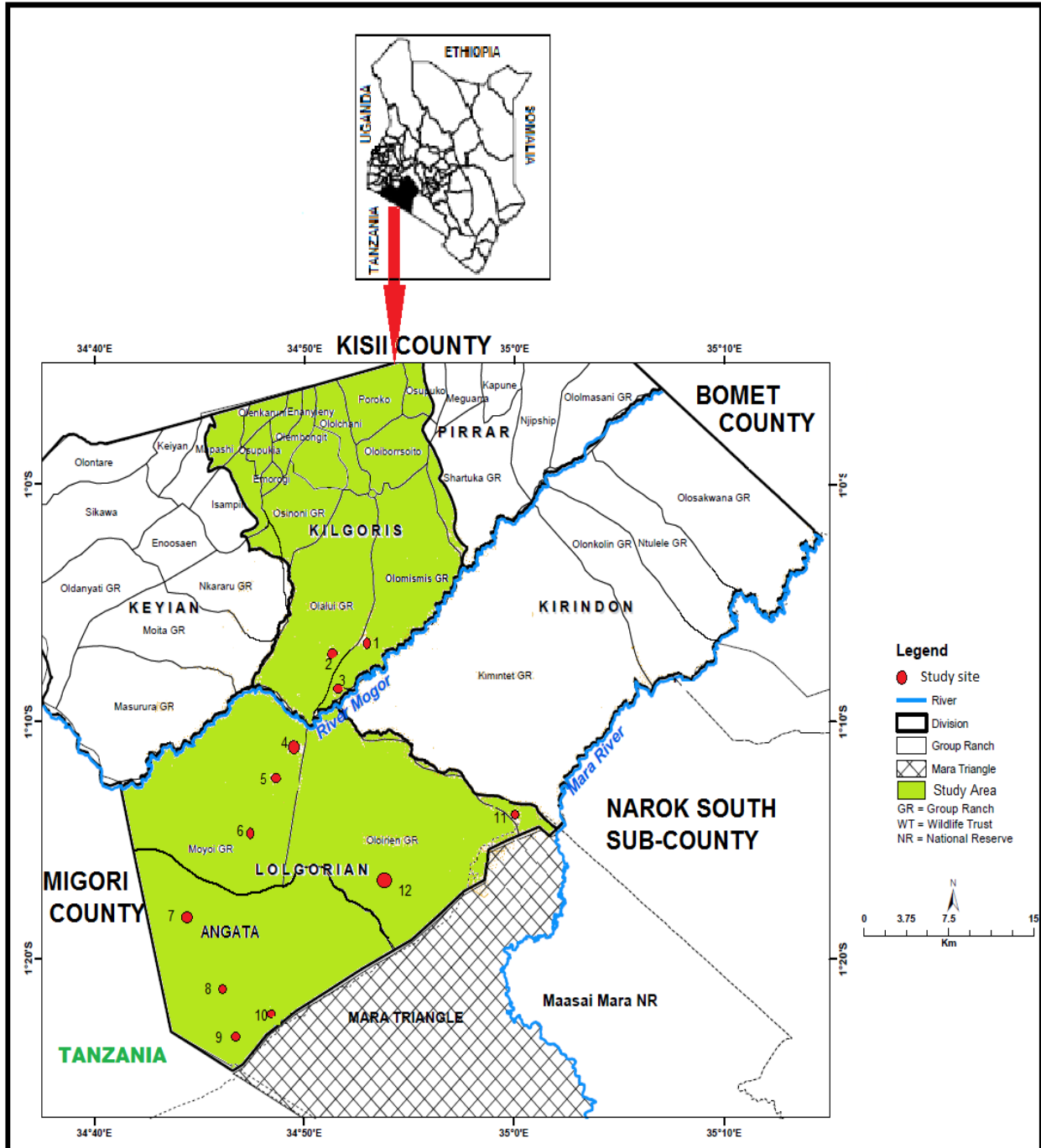
Figure 3.1. Transmara West Sub-County map indicating the study area (GOK, 2008).

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Details of the experimental study sites are indicated by numbers in the study area map (figure 3.1) besides the study site legend and described in table 3.1

Table 3.1. Experimental study sites location in the Transmara Sub-County study area

Site number in map (Fig. 3.1)	Site name	Division
1	Naserian	Kilgoris
2	Oloshomunyal	Kilgoris
3	Mongor	Kilgoris
4	Olmotonyi	Lolgorian
5	Olepoipoi	Lolgorian
6	Ololtingwal	Lolgorian
7	OLkiloriti	Angata
8	Oldonyoorok	Angata
9	Angata	Angata
10	Kondamet	Angata
11	Kawai	Lolgorian
12	KALRO Transmara Sub-centre	Lolgorian

3.3 Study Design

The research adopted longitudinal descriptive survey and experimental study designs. A survey was carried out to collect data from respondents on pesticides use patterns in Angata, Logorian and Kilgoris Divisions of Transmara West Sub-County. A list of beekeeping households in the sub-county (sampling frame) obtained from the sub-county Agricultural office was used to identify the households. The sampled households were selected using a random numbers table and pesticides use data collected using a structured questionnaire. Secondary data was obtained using a check list. In the experiment, a randomized complete block design (RCBD) was used to study the effects of pesticides use on honeybee mortality rate and honey production in Transmara West Sub-county. Sixteen apiaries were selected; five on-station and eleven on-farm for the experiments (Figure 1 and Table 1). All colonies in each apiary were evaluated for family

strength prior to start of the experiment using a standard method (Delaplane *et al*., 2013). This was done to help in selection of colonies with same family strengths to be used for the study.

Further, the study colonies were checked for the presence of Varroa mites using a standard sugar roll assay (Ellis and Macedo; 2001). A wide-mouth glass canning jar with two-piece lid; 8-mesh per inch hardware cloth to allow mites to pass through while retaining bees and one rounded teaspoon (7g) of powdered sugar were used. A circle of the hardware cloth was cut to fit inside the ring. Approximately 300 adult honeybees were collected in a wide mouth pint jar and powdered sugar was added through the hardware cloth. The jar was rolled to distribute the dust and coat the honeybees, let to sit for one minute, inverted and shaken over a white surface to recover mites. Any mites would pass through the screen while honeybees would remain in the jar. Further, the presence of small hive beetle, rats and wax moths were assessed by physical observation. In addition two main bacterial diseases affecting brood, the American foulbrood (AFB) and the European foulbrood (EFB) were assessed. Upon infection by AFB and EFB, the larvae exhibit a mosaic brood pattern of empty cells (dead larvae removed by nurse bees), uncapped cells with remains of diseased larvae and healthy capped cells in the infected colonies.

3.3.1 Experimental design

A randomized complete bock design (RCBD) was used to conduct the experimental study.

In the on-station experiment at KALRO Transmara Sub-Centre, three treatments and control were set up. Four 0.125 acre plots, two each on maize and beans, were established and two strong colonies managed in Langstroth hives transferred from the main station apiary and placed 20 metres from the maize and beans crop fields just after planting. Crop pests were managed

throughout their physiological stages including flowering stage by applying Magic (Malathion) and Keshet (Deltamethrin) pesticides according to the label instructions. Another two sets comprising of two strong colonies managed in Langstroth hives were transferred from the main station apiary and set 20 metres from a livestock spray crush at the KALRO Transmara Sub-Centre. Livestock were routinely sprayed with Sypertix (Alphacypermethrin), Almatix (Amitraz) and Steladone (Chlorfenvinphos) pesticides once a week to control external parasites after preparation according to the instructions on container label. At the main Station apiary, two apiaries were selected and two strong colonies managed in Langstroth hives identified in each apiary to serve as control experiment. The apiary was located at undisturbed natural vegetation, well fenced off and no farming or grazing ever takes place in it. It was guarded to ensure that no livestock grazed in or around it. Therefore no pesticides got to the colonies in the apiary either by drift or through transmission by grazing livestock.

Eleven on-farm apiaries, three in Kilgoris and four each in Angata and Lolgorian Divisions were selected for the study. In each apiary two strong colonies managed in Langstroth hives were identified. The colonies were constantly inspected for sanitary purposes and were monitored for honeybee mortality rates, honey yields and pesticides residues in honey, pollen and honeybees. All the treatments in the experiment were replicated three times over two seasons.

Dead bee traps were fixed at each hive entrances and mortality data collected at weekly intervals through two seasons. Honeybees, pollen and honey samples were collected from the hives containing the identified colonies and analyzed for pesticides residues using Queshers method (AOAC Official Method 2007.01) at SGS laboratories. A data sheet was designed for recording

colony seasonal honey yields data. The monitoring program carried out for two successive seasons: March - July 2015 and August - November 2015. Sample collection and pesticides residue analysis were done at the end of each season. Details of the sites, treatments, sampling units, sample sizes, matrices and units of analysis are summarized in table 3.2.

3.4 Monitoring honeybee Mortality rate and honey yields

Each hive containing the colonies under study was equipped with a collection cage for dead bees (an under basket trap collect dead honeybees). The under basket traps were considered to be the most suitable in retaining dead bees. The traps were attached to the hives seven days prior to the start of experiment to allow the honeybees time to adapt to the traps.

The hives were checked once a week and the number of dead bees were counted, recorded and removed. In those hives whose dead bees count exceeded the 250 critical threshold in an apiary, the dead bees were sorted and samples taken to laboratory for pesticides residue analysis. The dead bee traps were attached to the hives entrances on 16th March 2015 and remained fitted until 10th November 2016. The season one mortality rate data was collected from 6th April 2015 to 4th July 2015 while the season two mortality rate data was collected from 3rd August 2015 to 6th November 2015.

In addition, a data sheet was designed for recording honey yields data in each harvest season in all the colonies identified in the selected apiaries. Honey yields data was collected from 22nd - 26th June 2015 and 2nd- 6th November 2015 for seasons one and two respectively.

3.5 Sample collection

Season one honeybee's samples were collected between 6th April 2015 to 4th July 2015 while the season two honeybees samples were collected from 3rd August 2015 to 6th November 2015 at weekly intervals. Pollen and honey samples were collected from 22nd - 26th June 2015 and 2nd- 6th November 2015 for seasons one and two respectively.

3.5.1 Sampling honeybees for pesticides residue analysis

The identified colonies were checked weekly and dead bees removed from the traps, counted and sorted. Eight dead bee samples were taken and packed in a plastic jar, put in a cool box and stored at $4\ ^{0}C$ and subsequently analyzed for pesticides residues.

3.5.2 Sampling honey for pesticides residue analysis

Fifty (50) grams of freshly harvested honey from the hive containing the two strong colonies identified in all the sixteen selected apiaries were collected and packed in plastic jars and put in a cool box at $4\ ^{0}C$ and stored in a dark place, and later analyzed for pesticides residues.

3.5.3 Sampling pollen for pesticides residue analysis

Pollen samples were collected from comb cells of the two hives containing the strong colonies identified in all the sixteen apiaries. 20 grams of pollen from each colony was packed in plastic jar and stored in a cool box at $4\ ^{0}C$, in a dark place until their analysis. All the samples were taken to SGS laboratories for quantitative determination of pesticides residues.

3.6 Pesticides residue analysis

Honeybee, honey and pollen Matrices were preserved at 4^0C, extracted and analyzed following the modified QueCHers analytical method (AOAC Official Method 2007.01). The method uses a single-step buffered acetonitrile (MeCN) extraction and salting out liquid–liquid partitioning from the water in the sample with $MgSO_4$. Dispersive-solid-phase extraction (dispersive-SPE) cleanup was done to remove organic acids, excess water, and other components with a combination of primary secondary amine (PSA) sorbent and $MgSO_4$. The extracts were separated using chromatographic analytical separation and analyzed by mass spectrometry (MS) techniques.

3.6.1 Equipment

UPLC / MS-MS (Waters. Micromass Quattro Premier XE Mass Spectrometer) for quantifying the Amitraz and GC- MS (Agilent 7890A GC -5975C Inert MSD with Multi-mode Inlet) were used for other GC-amenable residues manufactured by Agilent (Karlsruhe, Germany) and an A J2-21M/E centifuge manufactured by Beckman (Rossy, France).

3.6.2 Reagents and consumables

Acetonitrile (Merck 1000302500- gradient grade for liquid chromatography or equivalent), Methanol (Merck 1000106035- hypergrade for LC-MS), Water (Merck 1000115333- For chromatography LiChrosolv), PSA Clean up Tube (Sigma 55282-U), $MgS0_4$ Extraction Tube (Sigma 55234-U) were obtained from Karlsruhe, Germany. Pesticide Pure standards (>99% certified purity) and Diethathyl Ethyl (an internal standard) were obtained from Dr Ehrenstrofer laboratory (Augsberg, Germany).

3.6.3 Pesticides residue analysis in honeybee

One g of honeybee heads was ground and mixed with 5 mL of 1% acetic acid (HOAc) in MeCN and 0.5 g anhydrous $MgSO_4$/NaOAc (4/1, w/w) and added to a centrifuge tube bottle, shaken and centrifuged. An upper layer portion of the MeCN extract was added to anhydrous $MgSO_4$/PSA sorbent (3/1, w/w; 200 mg per 1 mL extract), mixed, and centrifuged. The final extract was transferred to autosampler vials for analysis by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for identification and determination of pesticide residues in honeybee.

3.6.4 Pesticides residue analysis in honey

Two grams of honey was mixed with 5 mL of 1% acetic acid (HOAc) in MeCN and 0.5 g anhydrous $MgSO_4$/NaOAc (4/1, w/w) and added to a centrifuge tube. The mixture was shaken and centrifuged. A portion of the MeCN extract (upper layer) was added to anhydrous $MgSO_4$/PSA sorbent (3/1, w/w; 200 mg per 1 mL extract), mixed, and centrifuged. This final extract was transferred to autosampler vials for analysis by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for identification and determination of pesticide residues in honey.

3.6.5 Pesticides residue analysis in pollen

One gram of pollen sample was mixed with 2 mL of 1% acetic acid (HOAc) in MeCN and 0.5 g anhydrous $MgSO_4$/NaOAc (4/1, w/w) and added to a centrifuge tube, which was shaken and centrifuged. A portion of the MeCN extract (upper layer) was added to anhydrous $MgSO_4$/PSA sorbent (3/1, w/w; 200 mg per 1 mL extract), mixed, and centrifuged. This final extract was

transferred to autosampler vials for analysis by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) for identification and determination of pesticide residues in pollen.

3.6.6 Quality control

The precision of the method used in this study was established by analyzing samples in triplicate. The accuracy of the method was confirmed by running spiked honey, pollen and honeybee samples prior to actual sample analysis. This was further ensured by running blank solvents and standards (every six injections) between the injections. Blank solvents were run as an opportunity to evaluate and monitor the potential introduction of contaminants into the samples during processing. The agreement between the measured and certified concentrations of individual analyte confirmed the accuracy of the method. This reference material was introduced on regular basis after running 10 samples as a way of checking the procedure. The blanks were also introduced on a regular basis in between samples analysis. For recovery efficiency, 0.05 ppm of Amitraz, Malathion, Chlorfenvinphos, Alphacypermethrin and Deltamethrin standard mixture were added to 1g of pollen, 1g honeybee and 2g honey samples for analysis following procedures as indicated in subsections 3.4.4.3, 3.4.4.4 and 3.4.4.5. The Calibration curves for the LC-MS/MS & GC-MS were prepared from analytical standards at the following levels; 0 (matrix blank), 0.005, 0.01, 0.025, 0.05, 0.1 and 0.25 ppm. Diethathyl Ethyl was used as an Internal standard with a concentration 0f 0.1 ppm. The detection limits were found to be 0.005 ppm for Chlorfenvinphos and Cypermethrin while Amitraz, Metathion and Deltamethrin had a detection limit of 0.01 ppm.

Table 3.2. Summary of sites, treatments, sampling units and matrices in Transmara West Sub-county

Site	No of apiaries	No of colonies	Treatment	Monitoring pesticides residue				Monitoring mortality (threshold ≥ 250 dead h/bees/ apiary /week)		No of respondents
				Season 1 samples		Season 2 samples		Season 1 samples	Season 2 samples	
				honey	pollen	honey	pollen	honeybee	honeybee	
On-station (KALRO Transmara)	1	6	T1	1	1	1	1	1	1	
	1	6	T2	1	1	1	1	1	1	
	1	6	T3	1	1	1	1	1	1	
	1	2	C1	1	1	1	1	1	1	
	1	2	C2	1	1	1	1	1	1	
On-Farm			Farmer Practice							
Angata Division	4	24	Farmer Practice	4	4	4	4	4	4	115
Lolgorian Division	4	24	Farmer Practice	4	4	4	4	4	4	115
Kilgoris Division	3	24	Farmer Practice	3	3	3	3	3	3	100
SUB-Total	**16**	**94**		**16**	**16**	**16**	**16**	**8**	**8**	**330**
Total	**16**	**94**		**64**				**16**		**330**

n = 60 colonies; Treatments: T1 = Magic and Keshet, T2 =Almatix, Sypertix and Steladone, T3 = Magic, Keshet, Almatix, Sypertix and Steladone, C1 = Control1, C2 = Control 2, Farmer practice = Magic, Keshet, Almatix, Sypertix and Steladone

3.7 Study population

Transmara West Sub-County was estimated to have a human population of approximately 117,726 Persons and density of 62 persons per Km^2, with over 18,000 households. The number of households practicing beekeeping was approximately 2500 (ASDSP, 2013). A sample size of 330 respondents; livestock, crop, mixed farmers and beekeepers was selected randomly. They comprised 100 respondents in Kilgoris and 115 each in Angata and Lolgorian Divisions of Transmara West Sub-County (Krejcie and Morgan, 1970). The sample was distributed proportionately on the basis of the number of beekeeping households in each division. There are 752, 871 and 877 beekeeping households in Kilgoris, Angata and Lolgorian divisions respectively (GoK, 2008). Sixty colonies in sixteen apiaries; five at KALRO Transmara-station and eleven in farmers' fields; three in Kilgoris and four each in Angata and Lolgorian Divisions were selected for the study.

3.8 Sampling Procedure and Sample Size

Sampling procedure refers to a technique of selecting a part of population on which research can be conducted, which ensures that conclusions from the study can be generalized to the entire population. A sample in a research study refers to any group on which information is obtained. The researcher used cluster random sampling technique to select the respondents. The target population was 2500 beekeeping households. A sample size of 330 beekeeping households was selected for the study. This was determined using the Krejcie and Morgan (1970) table (Appendix 2) for estimating a sample size from a given population. The, sample was selected from each of the divisions based on the composition of the target population. The sample size was based on proportionate population distribution on target population in each division.

Enumerators were recruited from the study area and trained on the basic principles of conducting surveys and questionnaire administration. The selection of these enumerators from the area was important to help minimize language barrier and establish rapport among the respondents. The researcher and the trained enumerators pre-tested the questionnaire prior to full implementation of the data collection exercise with 30 selected respondents; ten from each division to check their understanding of the questions and to help improve on the tool in order to make it more effective in collecting the desired data. Respondents were drawn randomly from Lolgorian, Kilgoris and Angata Divisions (clusters) of Transmara West Sub-County from the sampling frame of 2500 beekeeping households using a random numbers table. The desired data collected from the respondents using a questionnaire (Appendix 1).

3.8.1 Data Collection Instruments

The study employed the use of a structured questionnaire to collect household pesticides use data. A check list was used to collect data from key informants while a simple data sheet was used to record honeybee mortality rate and honey yields data.

3.8.1.1 Questionnaire

This tool was developed by the researcher with the aid of the supervisors. The study preferred this tool because it can collect data from a large sample over a short period of time. This tool contained both open and closed ended question. Closed ended question are easy to analyze since they are in immediate usable form, easy to administer as each item is followed by alternative answer and are economical in terms of time and money. Open-ended questions stimulate a person to think about his/her feeling or motives and to express what he/she consider most vital

The questionnaire was administered to 115 beekeeping farmers each in Lolgorian and Angata divisions and 100 beekeeping farmers in Kilgoris Division. A checklist was prepared to gather information from agrochemical traders, Ministry of Livestock, Agriculture and Forestry staff. Secondary data was collected from existing reports of statutory regulatory bodies such as PCPB and NEMA. The data obtained using the checklist was meant to corroborate the one provided by farmers. The administered questionnaires were scrutinized to ensure that they were fully filled before data was entered in MS Excel spreadsheet software and statistically analyzed by SPSS version 16 software.

3.9 Validity and reliability of research instruments and methods

The research instruments for the study were tested for reliability and validity to ensure that they captured the aims and objectives of the research.

3.9.1 Validity of research instruments

Validity is the accuracy and meaningfulness of inferences, which are based on the research results. It is the degree to which results obtained from the analysis of data actually represent the phenomenon under study (Mugenda and Mugenda, 2003). To test the content validity of instruments, the researcher discussed the instruments with experts and specialists in Maseno University and KALRO to ensure that all the concepts under investigation were measured. A pilot study also aided in improving the validity of the instruments. Items were checked to ensure they accurately measured the concepts under study, were clear and understood by the respondents. In addition the experiments were conducted under standard conditions with

adequate replications. Samples collected from the experimental sites were subjected to standard analytical procedures with adequate controls analysed in triplicate and with blank solvents.

3.9.2 Reliability of the research instruments

The reliability of the instrument in the study area was done by pre-testing it through piloting. The exercise was conducted twice with same respondents. The responses were cross checked with respondents' next of kin most often the spouse or elder sons. Further samples collected were analysed using standard procedures with adequate quality control. The reliability of the items was based on the estimates of the variability among the responses to the items. The reliability coefficient was determined using Karl Pearson''s product moment correlation coefficient because the method was more accurate as it determined the stability of the instrument. The instruments were re-administered again to the same respondents after a period of two weeks and identification maintained. A reliability index alpha greater than or equal to 0.7 was considered to be high enough for the instrument to be used in the study (Mugenda and Mugenda, 2003).

3.10 Data analysis and presentation

Survey generated data was entered using MS Excel spreadsheet and analyzed using SPSS statistical software version 16. The mortality rate and honey yields data was analyzed using SAS software. Descriptive statistical summaries (95 % confidence interval, arithmetic means and standard deviations) were derived from the pesticides use patterns data. Significance level was accepted at $p < 0.05$. A one way ANOVA and Mean separation using Tukey's honestly significance test were used to establish the differences in the mortality rate and honey yields among the treated and control colonies.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the experimental and survey study results in tables, bar graphs, pie-chart and text. Moreover, the chapter presents the interpretation of the study findings and discussion in relation to the existing body of knowledge.

4.2 Effect of pesticides use on honeybee mortality and honey yields

4.2.1 Effect of pesticides use on honeybee mortality

The monitoring of honeybee mortality rates carried out in several sites in Transmara West Sub-County over two seasons, provided helpful information on the impact that crop and livestock protection management products used may have on honeybees. The results (Tables 4.1) indicate that there was no significant difference ($p = 0.089$) in mortality rates between the control and on-farm colonies in both seasons. The seasonal average honeybee mortality rate in the on-farm and control colonies were 77 ± 5.9 and 73 ± 12.0 respectively. However there was significant difference $(p = 0.01)$ in mortality rates between the control and treated colonies in both seasons. The season one average honeybee mortality rate in the control colonies was 64.0 ± 10 while that for the treated colonies were 229.00 ± 6.2, 231.00 ± 5.1 and 235 ± 4.3 in that order. The season two mortality rates had a similar pattern with the honeybee mortality rate in the control colonies being significantly different $(p = 0.01)$ from that of the treated colonies. The mortality rate for the control colonies was 82 ± 13 while that for the treated colonies were 228 ± 3.5, 230 ± 4.2 and 232 ± 3.8 in that order. That is the mortality rates in the treated colonies in both seasons were significantly higher than in the control and on-farm colonies (Season1: $MSD = 5.9655$; on-farm =

68.0 ± 6.1; control = 64.0± 10; treated = 232 ± 5.1; Season 2: *MSD* = 3.3919; on-farm = 85 ±

5.3; control = 82 ± 1.3, treated = 230 ± 5.1). Notably the mortality rates in the treated, control

and on-farm colonies were below the maximum threshold (250 dead bees per station per week)

to warrant further laboratory investigations (Porrini, 2003).

Table 4.1. Honeybee mortality rate on selected colonies in Transmara West Sub-County

	Mortality rate (No of dead bees per station per week) ± SE		
Treatments	Season 1	Season 2	Mean
On-farm	68.0 ± 6.1[b]	85 ± 5.3[b]	77 ± 5.9[b]
Control	64.0 ± 10[b]	82 ± 13[b]	73 ± 12.0[b]
Treatment1	229 ± 6.2[a]	228 ± 3.5[a]	229 ± 4.9[a]
Treatment2	231 ± 5.1[a]	230 ± 4.2[a]	231 ± 4.7[a]
Treatment3	235 ± 4.3[a]	232 ± 3.8[a]	234 ± 4.1[a]
MSD	5.9655	3.3919	4.6791
Mean	165	171	

n = 94 colonies; Means with the same superscripts within the column are not significantly different (Tukey test). Treatments: 1= Magic and keshet, 2=Almatix, sypertix and steladone, 3= Magic, keshet, almatix, sypertix and steladone.

The significantly higher mortality rates in the treated colonies than in the control and on-farm

colonies (Table 4.1) can partly be attributed to intense pesticides exposure among treated

colonies. This is because honeybees constantly forage in an area saturated with pesticides.

Similarly, the on-farm colonies have a higher chance of exposure to pesticides since farmers use

it to manage pests unlike the control colonies. These findings are consistent with Henry *et al*.,

(2012) and Teeters *et al*., (2012) findings. They observed that colonies located near treated crops

where most of their workers are exposed to pesticides in nectar throughout cropping seasons

could experience population decline from which the colonies will struggle to recover. The

finding that the mortality rates in the treated, control and on-farm colonies were below the

maximum threshold of 250 dead bees per station per week however, cannot be used to rule out honeybees' deaths due to poisoning by abuse and misuse of pesticides. This is because the impacts of pesticides on honeybees manifest themselves with differing degrees of severity in relation to various factors such as toxicity, time of application, application intensity and physiological maturity of flowers visited by bees (Sanford, 1993).

These results concur with PAN (2012) findings that concluded that honeybees near agricultural fields are exposed to a variety of pesticides via multiple routes at varying levels throughout the foraging period. Whereas high pesticides levels exposure to honeybees result in outright bees kill, sub-lethal doses on the other hand provoke behavioral difficulties such as loss of bees' navigation and communicative skills resulting in homing failure (Desneux *et al.,* 2007). Those that die instantly due to high dose exposure are not captured by the trap while bees exposed to sub-lethal doses tend to wander aimlessly due to loss of navigation ability. As a result the bees will not make it back to the hive hence the mortality rate count will be lower than expected due to the loss of foragers in the field (Sanford and Jamie, 2011).

Furthermore, concentrations of pesticides that are normally considered safe for honeybees in terms of individual acute toxicity have had a negative influence on their foraging behavior (Mommaerts *et al.*, 2010). For example honeybees exposed to pesticides at levels 70 times below the levels causing mortality in standard tests (LD_{50}) exhibited abnormal behavior such as inability to return to the nest (Colin *et al*. 2004). In addition, doses of deltamethrin as low as 2.5ng/bee (deltamethrin LD_{50} = 67ng/bee) were found to cause disorientation in foragers (Vandame *et al*., 1995). Thus although the mortality rate counts were within natural limits, it will

be inaccurate to conclude that pesticides use did not cause a substantial reduction in colony numbers in the study area. This was because some honeybees could have been lost in the field as a result of distorted navigation and homing abilities (Whitehorn *et al.,* 2012).

Although there is concurrence that pesticides use result in honeybee populations decline, the mortality rates are lower in the study area than in other regions. In North America and Europe for example, honeybee colony population have declined over the last 30 years, with beekeepers routinely reporting a 30 % loss of their managed colonies every winter during the last seven years (VanEngelsdorp and Meixner, 2010). Moreover up to 32 % of honeybees exposed to sub-lethal levels of pesticides failed to return to the hive in France, effectively doubling the natural loss rate of foraging workers (Henry *et al*., 2012). This can attributed to large pesticides quantities used and high application rates in Europe and USA (WenJun *et al*., 2011). In addition the acute, chronic and synergistic impacts of multiple pesticide exposures greatly contribute to declining honeybee health consequently increasing mortality rate (Johnson *et al.,* 2010).

4.2.2 Effect of pesticides use on honey yields

Monitoring of honeybee colonies for honey yields was carried out in several sites in Transmara West Sub-County over two seasons. There was evidence of significant difference *(p = 0.027)* in the yields for control colonies (18.0 ± 1.00 Kg) and on-farm colonies (12.20 ± 1.80 Kg) in season one. Similarly there was significant difference *(p = 0.019)* in the yields for control colonies (22.50 ± 1.50 Kg) and on-farm colonies (16.23 ± 2.05 Kg) in season two (Table 4.2). Moreover there was evidence that honey yields in the treated colonies were significantly lower than in the control colonies *(p = 0.024)* as presented in table 4.2. The average season one honey

yield in the control colonies was 18.0 ± 1.0 Kg while in the treated colonies were 7.1 ±1.10 Kg, 8.4 ±1.50 and 9.2 ±1.7 Kg in that order. The season two average honey yields in the control colonies was 22.5 ± 1.5 Kg while in the treated colonies were 11.0 ± 1.2 Kg, 15 ± 1.4 Kg and 13 ± 1.3 Kg respectively (Table 4.2).

Table 4.2. Honey yields on selected colonies in 2015 in Transmara West Sub-County

Treatments	Honey yields (Mean ± SE) Kg		
	Season 1	Season 2	Mean
On-farm	12.20 ± 1.80^b	16.23 ± 2.05^b	14.22 ± 1.93^b
Control	18.0 ± 1.00^a	22.50 ± 1.50^a	20.30 ± 1.3^a
Treatment1	7.1 ± 1.10^b	11.00 ± 1.20^b	9.0 ± 1.2^b
Treatment2	8.4 ± 1.50^b	15.0 ± 1.40^b	11.7 ± 1.5^b
Treatment3	9.2 ± 1.70^b	13.0 ± 1.30^b	11.0 ± 1.6^b
MSD	5.3431	4.3415	4.8425
Mean	10.98	15.55	

n = 94 colonies; Means with the same superscript within the column are not significantly different, at *p* = 0.05 (Tukey test). Treatments: 1 = Magic and keshet, 2 =Almatix, sypertix and steladone, 3 = Magic, keshet, almatix, sypertix and steladone.

The higher yields in the control than the treated colonies can be attributed to a higher mortality rate in treated colonies. This has the effect of reducing the number of foraging workers resulting in decreased honey yields. In addition, colonies that are exposed to pesticides tend to be weaker and cannot forage effectively hence lower honey yields in treated colonies (Vidau *et al*., 2011).

The results indicate that the average seasonal honey yield in the study area is 14 Kg/colony compared to 18 Kg/colony in the past (Carroll, 2002) hence consistent with other findings that honey production in the Sub-County and by extension Kenya has been declining. For instance the average annual honey production in 2005, 2006 and 2007 were 20.28 kg, 15 kg and 9.3

kg/colony in that order (NBS, 2007). Whereas beekeeping can be practiced with the highest

potential in dry areas where crop farming is not viable provided it is not in direct conflict or

competition with livestock rearing (Mutungi *et al*., 2003), it has been characterized by low honey

production in Kenya (Carroll, 2002). A number of studies have made the same observation

although they attributed the scenario to various constraints. Carroll (2002) attributed it to agro-

chemicals use, deforestation, drought and theft with pesticides being the greatest threat to the

enterprise. Mutungi *et al*., (2003) attributed competition between beekeeping and other

agricultural activities; cutting of trees and shrubs for construction, fencing and charcoal burning;

destruction of bee forage by caterpillar, poisoning of bees by pesticides as hindering honey

production to its potential in Kibwezi District. Kajobe *et al*., (2009) observed that one of the

most important factors that affect honey production was the multi-sectoral policy contradictions

and conflicts within the Ministry of Agriculture, Livestock, Industry and Fisheries for example

use of agricultural chemicals. Thus it was evident from these studies that pesticides use result in

decline in honey yields.

4.3 Pesticides residues in honeybee, honey and pollen

Pesticide analysis was performed on honey, honeybee and pollen samples from 94 colonies in 16

apiaries. Samples were screened for five pesticides; Amitraz, Chlorfenvinphos, Malathion,

Cypermethrin and Deltamethrin. This was because; these pesticides are mostly used in the study

area. Besides, they belonged to moderately hazardous class except Chlorfenvinphos (highly

hazardous) (WHO, 2010). The detection limits were found to be 0.005 ppm for Chlorfenvinphos

and Cypermethrin while Amitraz, Malathion and Deltamethrin had a detection limit of 0.01 ppm

(Table 4.3). The recoveries of spiked samples ranged from 87 % to 94 % that were above the

acceptable range > 70 %. However, no pesticides residues were detected in all the samples (Table 4.3).

Table 4.3. Analytical results of honey, pollen and honeybee samples in Transmara West Sub-County

Matrix	Pesticide trade name	Active ingredient (A.I)	Pesticides levels in sample (mg/Kg)	Limit of detection (mg /Kg)	Limit of quantification (mg /Kg)	Percentage (%) recoveries
Honey	Almatix	Amitraz	< LOD	0.01	0.01	90 ± 3.0
	Magic	Malathion	< LOD	0.01	0.05	87 ± 5.7
	Steladone	Chlorfenvinphos	< LOD	0.01	0.05	94 ± 3.7
	Sypertix	Cypermethrin	< LOD	0.01	0.01	92 ± 4.3
	Keshet	Deltamethrin	< LOD	0.01	0.01	93 ± 1.5
Pollen	Almatix	Amitraz	< LOD	0.01	0.01	90 ± 3.0
	Magic	Malathion	< LOD	0.01	0.05	87 ± 5.7
	Steladone	Chlorfenvinphos	< LOD	0.01	0.05	94 ± 3.7
	Sypertix	Cypermethrin	< LOD	0.01	0.01	92 ± 4.3
	Keshet	Deltamethrin	< LOD	0.01	0.01	93 ± 1.5
Honeybee	Almatix	Amitraz	< LOD	0.01	0.01	90 ± 3.0
	Magic	Malathion	< LOD	0.01	0.05	87 ± 5.7
	Steladone	Chlorfenvinphos	< LOD	0.01	0.05	94 ± 3.7
	Sypertix	Cypermethrin	< LOD	0.01	0.01	92 ± 4.3
	Keshet	Deltamethrin	< LOD	0.01	0.01	93 ± 1.5

$n = 80$ matrices; <LOD = below limit of detection

The results indicate that there were no pesticides residues detected in all the matrices. This can be attributed to the degrading nature of pesticides over time as they interact with the environment (Greig-Smith *et al*., 1994). Additionally, honeybees degrade pesticides following exposure through their gut filtering mechanism (Schur and Wallner, 2000). Honeybees ingest most of the chemicals just after exposure, and then rapidly eliminate it by metabolism, advection and deposition hence reducing the initially high pesticides concentrations (Tremolada *et al*. 2004).

These findings are consistent with findings of previous studies in the region. Orina (2012) found that there were no pesticides residues in all the honey samples collected from different sites from 13 regions throughout Kenya and analyzed for pesticides residues in the laboratory. Muli *et al*., (2014) performed pesticide analysis on honey samples from 13 sites across Kenya by screening for the presence of 171 pesticides. Only four pesticides; 1-naphthol, Chlorothalonil, Chlorpyrifos and fluvalinate were detected mostly at very low levels (below 50 ppb). Further Bogdanov *et al*., (2003) screened 27 honey samples produced in Switzerland for 36 organochlorine, 32 organophosphorous pesticides and six fungicides and found no pesticides residues. This can be attributed partly to low application rates of pesticides in Kenya and a strict pesticides regulations in Switzerland (FAO, 2010; WenJun *et al*., 2011). Therefore it is possible to effectively control agricultural pests with pesticides while maintaining the environmental integrity (Kasina, 2012). However, these results contrast findings from other studies globally that reported residues in honey, pollen and honeybee. For example over 90% of honeybee colonies in the USA contained pesticide residues with over 129 different pesticide-related chemicals being found, with an average of six chemicals per colony (Mullin *et al*., 2010). Cristina *et al*., (2003) analyzed honey samples from Portugal and Spain and found that most were contaminated with organochlorine pesticides with Portuguese honeys being more contaminated than Spanish ones.

The differences in residues can be attributed to different volumes consumed and application rates of pesticides (Reus *et al*., 2000). For instance USA, Brazil and Spain consume 0.4, 0.12 and 0.11 million tons annually in that order (FAO, 2010; WenJun *et al*., 2011). In addition Spain emphasizes on pesticide applicator training, considered one of the most relevant aspects in the reduction of pesticide exposure and consequently their honeybee products are less contaminated

than Brazil ones (Tremolada *et al.* 2004). Moreover Kolankaya *et al.*, (2002) detected Aldrin residues in six honey (very low levels) and two pollen samples. He further detected Carbosulfan and Carboryl pesticides residues in dead honeybees' heads in Ankara, Turkey while Maja *et al.*, (2010) found Fluvalinate in bee heads after external doses of pesticides were applied to colonies of nine combs, occupied with 20,000–30,000 adult honeybees located at the agricultural institute of Slovenia.

The low pesticides residues levels in honey compared to honeybee or its other products can partly be attributed to a filtering effect of bees (Schur and Wallner, 2000). He observed that indeed, honeybees decreased initially high pesticide nectar concentrations so that the final concentration in honey was much lower, mostly by a factor of about 1000. Additionally, bees immediately begin to degrade pesticides following exposure, further reducing concentrations of remaining pesticide residue (Greig-Smith *et al.*, 1994 and Tremolada *et al.* 2004). Therefore the low levels of pesticides in honeybee products from across Kenya, particularly when compared to levels in developed countries, suggests pesticide consumption is low and that they impact minimally on honeybee health at present in Kenya (Muli *et al.,* 2014).

4.4 Survey results on pesticide use patterns among farmers in Transmara West Sub-County
The survey assessed the profile of 330 farmers. The results (Table 4.4) indicate that 60 % of the respondents are male, mostly adults aged between 25 and 50 years of age. The area has a low literacy level with illiterate and primary education levels accounting for 35 % and 34 % respectively. Secondary, tertiary and university education, which are indicators of high literacy only accounts for 13 %, 2 % and 1 % in that order while 15 % had informal education.

Table 4.4. Farmers' profile: Age and education level in Transmara West Sub-County

| | Angata | | Kilgoris | | Lolgorian | | Mean |
	Frequency	%	Frequency	%	Frequency	%	%
Age							
< 18 years	0	0	19	19	9	8	8
18-35 years	50	44	44	44	53	46	45
35-50 years	36	31	27	27	46	40	33
> 50 years	29	25	10	10	7	6	14
Level of education							
Illiterate	25	22	41	41	50	43	35
In-formal	14	13	20	20	13	11	15
primary	58	50	32	32	22	19	34
Secondary	14	13	7	7	22	19	13
Tertiary	4	2	0	0	4	4	2
University	0	0	0	0	4	4	1

n = 330

The level of illiteracy was lower in Angata compared to the other divisions (Table 4.4). This can be attributed to the migrant community living in the division that came from a literate background and hence mobilized resources and established schools earlier in their area. These results are comparable to Kenya's adult population illiteracy level of 38.5% with notable disparities between various regions and across gender (KNBS, 2007, Berem, 2009). However it was contrary to Nyeri's high literacy level with 76% having secondary education (Gitahi, 2014). This was despite education being a tool for promoting development of any country (Mwaluko, 2009). Since education level is correlated with pesticides handling, the low levels of education in developing countries must be improved for proper use of pesticides to be met (Wandiga, 2001).

Majority of the residents keep cattle, goats and sheep at 98 %, 94 % and 89 % in that order (Table 4.5). More than half of the population keeps chicken (55 %) while 22 % keep donkeys.

The most popular crops among the farmers in the three divisions were maize and beans grown by 70 %, and 52 % of the farmers respectively. Others are kales (26 %) and tomatoes (15 %).

Table 4.5. Livestock kept and crops grown in Transmara West Sub-County

	Angata		Kilgoris		Lolgorian		Mean
Livestock	Frequency	%	Frequency	%	Frequency	%	%
Cattle	115	100	95	95	114	99	98
Goats	115	100	85	85	111	96	94
Sheep	104	90	83	83	106	92	89
Chicken	61	53	51	51	71	62	55
Donkey	47	41	17	17	13	11	22
Crops							
Maize	110	96	61	61	60	52	70
Beans	92	80	36	36	45	39	52
Kales	58	50	10	10	17	15	26
Tomatoes	22	19	5	5	23	20	15

n = 330

Angata farmers constituted a higher proportion of bean growers (88 %), Lolgorian (24 %) and Kilgoris (17 %) since it is grown as an intercrop with maize. The economic activities in the study area were comparable to some parts of the country. Nyeri farmers grew; maize, beans and vegetables among others but kept dairy cattle (Booker *et al*, 2009; Gitahi, 2014). Cattle, goats and sheep were kept while maize, beans and vegetables were grown in Laikipia and Isiolo, with 69 % of farmers growing maize (Otieno *et al*., 2010).

From the survey results, it was found that livestock keeping was a major activity for most of the households (89 %) while the rest engaged in other activities such as crop farming, trade and formal employment (Table 4.6). The mobility of the livestock in the study area is essentially sedentary (98 %). The rest comprised nomadic livestock keeping. Herding was the main mode

of grazing livestock among the households (88 %) while 10 % free graze. A small proportion (2 %) put their livestock in paddocks.

Table 4.6. Importance, mobility and grazing of livestock in Transmara West Sub-County

Livestock activity	Angata		Kilgoris		Lolgorian		Mean
	Frequency	%	Frequency	%	Frequency	%	%
Major	90	78	98	98	107	93	89
Minor	25	22	2	2	8	7	11
Mobility							
Sedentary	114	99	98	98	113	98	98
Nomadic	1	1	2	2	2	2	2
Grazing							
Herding	83	72	100	100	106	92	88
Free grazing	28	24	0	0	6	5	10
Paddock	4	4	0	0	3	3	2

n = 330

Notably the main mode of grazing in the area was herding. This can be attributed to the vast fallow land mass; hence farmers can afford to graze their livestock widely without causing conflict among their neighbours. Sedentarization has been a worldwide phenomenon with, formerly nomadic livestock-keeping pastoralists settling in many parts of the world within the past one century (Roth and Fratkin, 2005). The pastoral communities settled mainly in response to ecological decline or new market opportunities. For example, the Maasai community settled near roads and urban areas like Nairobi for easy access to cattle markets, while in Northern Kenya, the Borana settled on Marsabit Mountain so as to provide beef and milk to police posts and road crews (Roth and Fratkin, 2005). Today, mobile pastoralists in Eastern Africa are becoming sedentary due to population pressure, droughts and famines (Ekaya, 2001). However,

sedentarization brings about negative ecological impacts due to intense localized utilization of vegetation with high value species like *Acacia spp.* being overexploited (Ekaya, 2005).

The results further indicate that ticks are the external livestock parasite of most economic importance according to 31 % of the sample population while 26 % reported tsetse flies are a big threat to their livestock, a main livelihood (Table 4.7). Other livestock parasites were; ticks, mites and tsetse (16 %), tsetse and worms (14 %) and ticks and worms (13 %). Stem borer was a major maize pest of economic importance according to 40 % of the farmers. Another 24 % of the farmers reported aphids caused them heavy economic losses. Bean flies infested 12 % of the farms while nematodes were reported by nine percent of the farmers. Further seven percent stated that army worms infested their crops while four percent of the farmers stated that stem borers and bean flies infested their crops.

Table 4.7. Livestock parasites and crop pests in Transmara West Sub-County

	Angata		Kilgoris		Lolgorian		Mean
Livestock parasites	Frequency	%	Frequency	%	Frequency	%	%
Ticks	36	32	25	25	42	37	31
Tsetse flies	22	19	10	10	55	47	26
Ticks, mites, tsetse	18	16	15	15	19	17	16
Tsetse & worms	14	12	24	24	8	7	14
Ticks & worms	13	10	12	12	17	16	13
Crop pests							
Stem borer	36	31	49	49	47	41	40
Aphids	36	31	24	24	19	16	24
Bean flies	14	13	9	9	17	15	12
Nematodes	11	10	8	8	11	10	9
Army worm	7	6	5	5	7	6	7
Stem borer & beanfly	4	3	2	2	8	7	4

n = 330

Distinctly most farmers could recognize livestock pests by name than crop pests probably due to the areas livestock background. Hence there is need for suitable training to build the capacity of farmers to identify the common pests and diseases for their crops (Sithanantham, 2004). This is because pest management approaches have always succeeded where farmers recognize the pest problem as a production constraint (Heong and Escalada, 1999; Joshi *et al.*, 2001).

The predominant method for controlling external livestock parasites and crop pests in the area was use of chemical pesticides (91 %) indicated in (Figure 4.1). The specific pesticides used are indicated in table 4.8. Four percent of the population burned dry grass in the grazing fields to control ticks. The burning of dry grass destroys the ticks' breeding areas. Three percent use ethno drugs, while a paltry two percent practice manual parasites picking whenever they were spotted on their livestock skins. This applied to those with a few number of livestock.
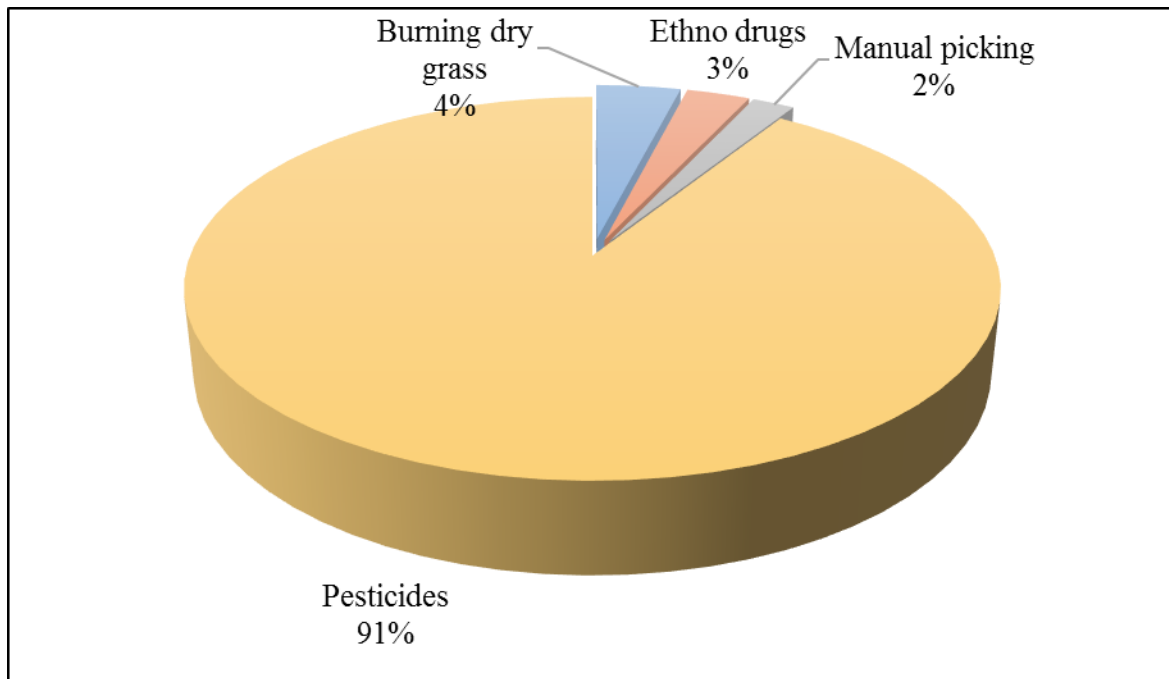
Figure 4.1. Pests control methods used in Transmara West Sub-County (%)

The choice of pest control method (pesticides) was influenced by cost, accessibility and efficacy (GoK, 2008). These findings are consistent with those of past studies. For example Sibanda *et al.*, (2000) observed that farmers choose pest management options that appear to meet their objectives based on their beliefs towards damage and control. Pesticides are often used as the primary control method in agriculture because of their convenience and cost effectiveness (Mutuku *et al.*, 2013). However, they cause environmental contamination, hence the interest in adoption of crop rotation, resistant varieties, cultural practices, and biological controls as the first line of defense (Kasina, 2012). This requires producers to plan for their use in advance of pest outbreaks to successfully use nonchemical management tools (Nderitu *et al.*, 2007).

About twenty different types of pesticides were used in the study area to combat crop and livestock pests. Pyrethroids accounted for 50 %, while organophosphorous and formamidine each accounted for 25 % of the total pesticides used in the study area (Table 4.8). The number of farmers using individual products (Table 4.8) indicated that Amitraz and Cypermethrin were most frequently used accounting for 73 % and 59 % respectively of all pesticides. Others were; Malathion (27 %), Diazinon (26 %), Deltamethrin (25 %), Chlorfenvinphos (10 %) and Cyhalothrin (7 %) of the total pesticides used by farmers to control pests in the area (Table 4.8). All pesticides used in the area were duly registered in Kenya except Cybadip. Most farmers chose pyrethroids compared to others partly due to their affordability, accessibility and efficacy.

Table 4.8. Pesticides used by livestock and crop farmers in Transmara West Sub-County

Pesticide chemical group	Pesticide Trade name	Active ingredient (A.I)	WHO toxicity class	Registration status (PCPB)	Farmers using (%)
Formamidine	Almatix	Amitraz	II	Registered	35
	Bye bye	Amitraz	II	Registered	4
	Norotraz	Amitraz	II	Registered	18
	Tixfix	Amitraz	II	Registered	14
	Triatix	Amitraz	II	Registered	2
Organophosphorous	Diazol	Diazinon	II	Registered	6
	Neocidol	Diazinon	II	Registered	20
	Magic	Malathion	II	Registered	15
	Oshothion	Malathion	II	Registered	12
	Steladone	Chlorfenvinphos	Ib	Registered	10
Pyrethroids	Alfapor	Cypermethrin	II	Registered	5
	Alphacymba	Cypermethrin	II	Registered	13
	Dominex	Cypermethrin	II	Registered	2
	Sypertix	Cypermethrin	II	Registered	28
	Cybadip	Cypermethrin	II	Not Registered	5
	Ectomin	Cypermethrin	II	Registered	6
	Grenade	Cyhalothrin	II	Registered	7
	Delete	Deltamethrin	II	Registered	4
	Keshet	Deltamethrin	II	Registered	16
	Vectocid	Deltamethrin	II	Registered	5

$n = 330$, Toxicity classes: Ia = Extremely harzardous, Ib = Highly harzardous, II = moderately harzardous, III = slightly harzardous (WHO, 2010).

These results are consistent with findings of past studies. Williamson et *al*., (2008) found that chemical pest control was the dominant strategy with about 47 different pesticide active ingredients reported by farmers. Further, Macharia et *al*., (2009) found 62 products, comprising of 36 active ingredients were used in vegetable production in Kenya, with a higher volumes of organophosphates than pyrethroids class. This was the case among vegetable growers in Eastern Africa (Sithanantham, 2004). This variation was attributed to the target crop or livestock pests.

The encounter of unauthorized and highly hazardous pesticide use (WHO, 2010) although not widespread was generally an extremely hazardous practice (Sibanda *et al*., 2000; Dinham, 2003).

The majority of livestock farmers (79 %) spray their livestock weekly, 19 % biweekly while 2 % spray on a monthly basis (Table 4.9). Farmers spray their livestock with pesticides during various times of the day with 93 % applying during morning hours. About 40 % spray early in the morning at 6am-8am, 53 % spray at 8 am -10 am while seven percent sprayed between 10 am and noon. No farmers sprayed their livestock in the afternoon and evening (Table 4.9).

Table 4.9. Pesticides use intensity among livestock farmers in Transmara West Sub-County

	Angata		Kilgoris		Lolgorian		Mean
Frequency of use	Frequency	%	Frequency	%	Frequency	%	%
Weekly	90	78	83	83	87	76	79
Biweekly	23	20	15	15	25	22	19
Monthly	2	2	2	2	3	2	2
Application time							
6-8 am	43	37	42	42	47	41	40
8-10 am	68	59	42	42	64	56	53
10-12 noon	4	3	16	16	4	3	7
Afternoon	0	0	0	0	0	0	0
Evening	0	0	0	0	0	0	0

n = 330

The lack of awareness and safety insensitivity among farmers on the honeybees' activity seems to inform their livestock spraying schedule with pesticides that is in direct conflict with honeybees (Table 4.9). This was because honeybees are most active from 8 a.m. to 5 p.m. (Sanford and Jamie, 2011) hence high likelihood of pesticides exposure. This also confirms the concern by the PCPB that environmental health problems associated with pesticide application

are usually blamed on the pesticides without considering how they were applied (PCPB, 2005; Williamson *et al*., 2008).

Majority (63 %) of the farmers applied pesticides to their crop fields weekly, 26 % spray biweekly while 10 % apply monthly (Table 4.10). The pesticides were applied mostly in the morning with 36 % of them spraying at 6-8 am, 42 % spray at 8-10 am, 16 % spray at 10-12 noon while 5 % spray in the afternoon with none spraying in the evening. This was the case throughout all the crops physiological including all flowering stages (Table 4.10).

Table 4.10. Pesticides use intensity among crop farmers in Transmara West Sub-County

	Angata		Kilgoris		Lolgorian		Mean
Frequency of use	Frequency	%	Frequency	%	Frequency	%	%
Weekly	77	67	64	64	68	59	63
Biweekly	25	22	20	20	30	26	22
Monthly	9	8	9	9	16	14	10
Application time							
6-8 am	39	25	46	46	35	30	36
8-10 am	56	49	42	42	42	36	42
10-12 noon	20	17	12	12	21	18	16
Afternoon	0	0	0	0	18	16	5
Evening	0	0	0	0	0	0	0

n = 330

There is limited awareness and knowledge among farmers on the negative impact of pesticides application on the environment and honeybees in particular (Table 4.10). These results also imply that majority of the crop farmers will have sprayed their crops 2-12 times in a single growing season indicating that pesticides use in crop pests control is haphazard and confirms findings of other studies. Pesticides use has been reported to be widespread among farmers in

some countries. Sibanda *et al.,* (2000), reported that several sprays were applied in every growing season in Zimbabwe. Nderitu *et al.,* (2007), observed that Kenyan farmers applied insecticides up to 15 times during a single cropping season for crops such as French beans. This disregard to time and stages of plant growth while spraying crop fields with pesticides exposes honeybees to hazardous pesticides and is inconsistent with the recommendation that pesticides must be applied to blooming plants when bees are not working, preferably in the early evening (Kolankaya *et al.,* 2002). This allows time for these chemicals to partially or totally decompose during the night. It is recommendation that insecticides should be applied only while target plants are in the bud stage or just after the petals have dropped (Sanford and Jamie, 2011).

The survey showed that farmers acknowledged negative pesticides effects on honeybees and the whole beekeeping enterprise, with varying degrees of severity (Figure 4.2). Over 52 % of the respondents cited Dominex as most responsible for honeybee colony decline. About 40 % of the farmers believed that Sypertix was the most severe while 33 % of the respondents believed that Alfapor caused colony decline. Another 29 % and 28 % of the beekeepers respectively thought that Alphacymba and Cybadip were responsible for the malady. Steladone and Delete each contributed to bees decline according to 21 % of farmers while 17 % blamed it on Oshothion. Malathion had the least effect at 13 %, meaning it does not affect honeybees as much.
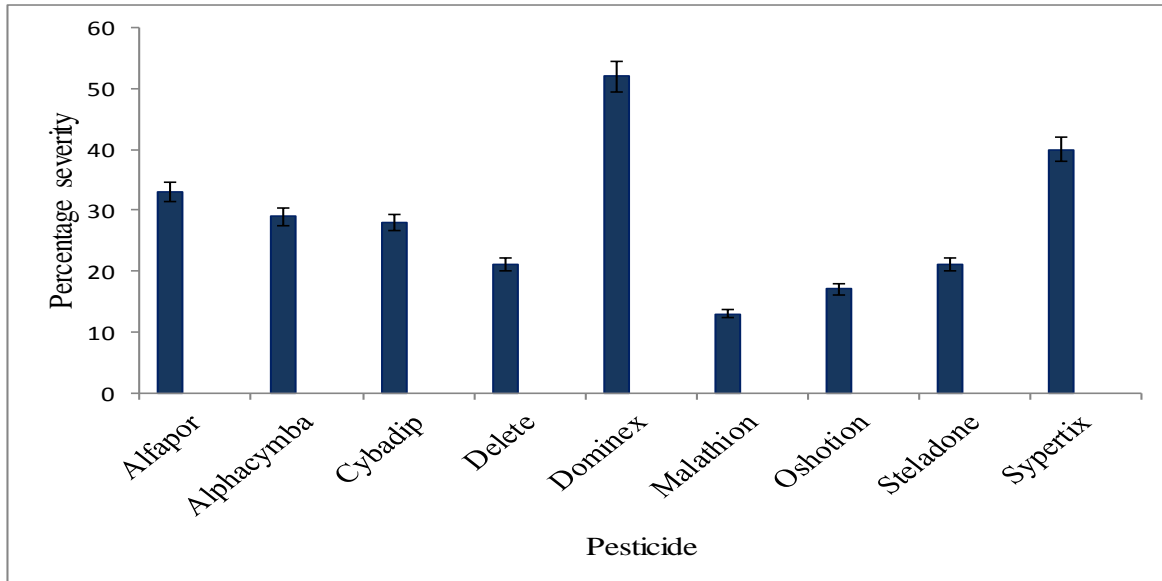
Figure 4.2. Severity of pesticides to honeybees in Transmara West Sub-County

The high Dominex and Sypertix impact on honeybees was due to their extensive use in tsetse fly eradication campaign besides control of ticks. Farmers threat tsetse flies and would use higher concentrations of pesticides solutions to ensure they got eradicated. Cases of pesticides poisoning have been reported around the world with varying degrees of severity. In Benin, pesticides containing Chlorpyrifos and lambda-Cyhalothrin have caused ill health episodes with about 47 % of villagers being adversely affected each season (Williamson *et al*., 2008). While in East Africa, pesticides poisoning have been proved to cause declines in honeybee colony populations (Musimba *et al*., 2001; Kajobe *et al*., 2009). This was due to inappropriate use contrary to manufactures recommendations thus pesticides must be handled with care to ensure pests control while conserving the environment (Nderitu *et al*., 2007; Kajobe *et al.,* 2009).

The major information sources among farmers' on pesticides use were; neighbouring farmers (51 %), friends (16 %), agro-dealers (12 %), and media (10 %) while eight percent was introduced

by extension staff. On reliability of information source among farmers, extension staff, leading farmers and media were most trusted at 39 %, 37 % and 17 % in that order (Table 4.11).

Table 4.11. Farmers' information sources on pesticides in Transmara West Sub-County

Introduction to pesticides use	Angata		Kilgoris		Lolgorian		Mean
	Frequency	%	Frequency	%	Frequency	%	%
Neighbours	62	54	50	50	56	49	51
Friends	12	10	20	20	21	18	16
Agro dealers	14	12	7	7	17	15	12
Media	7	6	10	10	16	14	10
Extension	11	10	7	7	8	7	8
Trust							
Extension	54	46	35	35	39	34	39
Leading farmers	34	30	48	48	40	35	37
Media	25	22	17	17	13	11	17

n = 330

Most farmers had inadequate access to reliable sources of pesticides information, probably due to inadequate extension coverage. The results are consistent with findings from past studies such as Sithanantham (2004) who found that farmers did not have adequate access to IPM information and depended heavily on neighbours and agrodealers. PAN (2012) found that state extension services in countries such as Uganda were unable to provide adequate coverage and information to the public. Inappropriate pesticides use has always been blamed on the inadequate extension services due to staff shortage and inadequate training resources (Ngowi *et al*., 2007).

A significant proportion (78 %) of farmers sprays pesticides to their livestock at spray races in their homes to control pests (mainly ticks) (Table 4.12). While 34 % use a single type of pesticides, 66 % use a cocktail of pesticides. Over 84 % of the farmers were supplied with

pesticides by registered agro dealers. Seven percent each obtained their pesticides supplies from their local kiosks and middlemen while two percent obtained their supplies from extension staff.

Table 4.12. Pesticides' suppliers and use patterns in Transmara West Sub-County

	Angata		Kilgoris		Lolgorian		Mean
Dipping	Frequency	%	Frequency	%	Frequency	%	%
Yes	43	37	7	7	22	19	22
No	72	63	93	93	93	81	78
pesticides used							
One	39	34	44	44	28	24	34
More	76	66	56	56	87	76	66
Reasons for >1							
Efficacy	115	100	100	100	115	100	100
Suppliers							
Agro dealer	102	89	76	76	98	87	84
Kiosk	5	4	10	10	8	7	7
Middlemen	5	4	12	12	7	6	7
Extension office	3	3	2	2	2	2	2

n = 330

The study revealed that cattle dips use has tremendously declined owing to the accessibility of pesticides by farmers spraying livestock in their spray crushes. This was despite dipping being Kenyan government's tick control policy (GoK, 2008). These findings are consistent to others. There have been reports of widespread pesticides use often sourced from unauthorized dealers, selling products of dubious quality (Williamson *et al*., 2008). The use of a cocktail of pesticides was intended to achieve efficacy due to synergy associated with mixing two products serving the same function (Gitonga *et al*., 2008). Although farmers were often aware of quality problems in non-authorized channels they felt the advantage of accessibility outweighed the risks of being sold adulterated products (Macharia *et al*., 2009). Use of pesticide cocktails was reported by vegetable farmers in Ethiopian, Benin and Kenya (Williamson *et al.*, 2008; Mutuku *et al*., 2013).

The study found that only 24 % of the farmers checked container label for safety reasons (Table 4.13). Over 67 % of the farmers applied pesticides using Knapp sack sprayers while 33 % used hand sprayers. About 29 % of the respondents put on protective clothing while handling pesticides. Most farmers (59 %) stored pesticides in their granaries, 21 % in pesticides stores while 20 % stored them in their living rooms. About 17 % threw empty containers into pit latrines, 58 % threw them away while 25 % poured expired pesticides haphazardly.

Table 4.13. Pesticides quality control and safety measures in Transmara West Sub-County

Check container label	Angata		Kilgoris		Lolgorian		Mean
	Frequency	%	Frequency	%	Frequency	%	%
Yes	18	16	24	24	36	31	24
No	97	84	76	76	79	69	76
Pesticides application							
Hand spraying	34	30	32	32	42	37	33
Knapsack	81	70	68	68	73	63	67
Wear protective gear							
Yes	43	37	22	22	30	26	29
No	72	63	78	78	85	74	71
Storage							
Granary	93	81	49	49	54	47	59
Living room	4	3	34	34	28	24	20
Pesticide store	18	16	17	17	33	29	21
Disposal							
Pouring	14	12	53	53	17	15	25
Throwing away	64	56	58	58	69	60	58
Pit latrine	36	31	5	5	14	12	17

n = 330

The findings revealed that while handling pesticides, farmers exposed their health and the environment to serious risks mainly due to indifference to safety issues and concur with findings

of past studies. For example lack of protective clothing has been found to cause eye and skin irritations (Gitahi, 2014). Storing pesticides in granaries and living room may spill in food or get easily accessible to children and cause chemical poisoning (Williamson *et al*., 2008; WHO, 2009). In addition, the disposal process is wanting as the pesticides residues get into the environment through run-off during the rainy season contaminating water bodies hence affecting organisms such as fish (Otieno *et al.,* 2010; Gitonga *et al*., 2010). This is one of the ways through which honeybees are exposed to pesticides. This will contaminate stagnant water that bees may drink resulting in their death or for their brood (Sanford and Jamie, 2011).

Over 82 % (270) of the respondents are beekeepers owning log hive (91 %); Langstroth (5 %) and KTBH (4 %) (Table 4.14). About 14 % of them have practiced beekeeping for less than five years, while 20 %, 50 % and 16 % have practiced it for; 5 -10 , 11- 20 and over 20 years in that order. The results indicate that majority were reasonably experienced beekeeping.

Table 4.14. Beekeeping practices in Transmara West Sub-County

Practice beekeeping	Angata		Kilgoris		Lolgorian		Mean
	Frequency	%	Frequency	%	Frequency	%	%
Yes	90	78	85	85	95	83	82
No	25	22	15	15	20	17	18
Hive type							
Loghive	76	84	85	100	86	91	91
Langstroth	7	8	0	0	6	6	5
KTBH	7	8	0	0	3	3	4
Beekeeping period							
1-5 years	14	16	10	12	13	14	14
5-10 Years	11	12	20	23	23	24	20
11-20 Years	50	56	38	45	48	50	50
> 20 years	15	17	17	20	11	12	16

n = 330

In addition they confirm that log hives remain popular in honey production in the study area probably due to ease of accessibility and costs. This finding concurs with other findings for example Musimba *et al*., (2001) and Carroll (2002) found that despite concerted efforts being made to promote Langstroth and KTBH hives, log hives remained the hive of choice for most beekeepers. This was partly attributed to higher initial capital needed to acquire the modern hives although they come with the advantage of ease of operation (Kajobe *et al*., 2012).

Notably all the respondents irrespective of their occupation variedly concurred that honeybees are of major economic importance. About 83 % stated that honeybee products serve as food; medicinal value (82 %), raw material for alcohol (67 %) and industrial use (60 %) while 64 % stated honeybees play role in cross pollination and biodiversity conservation (Figure 4.3).
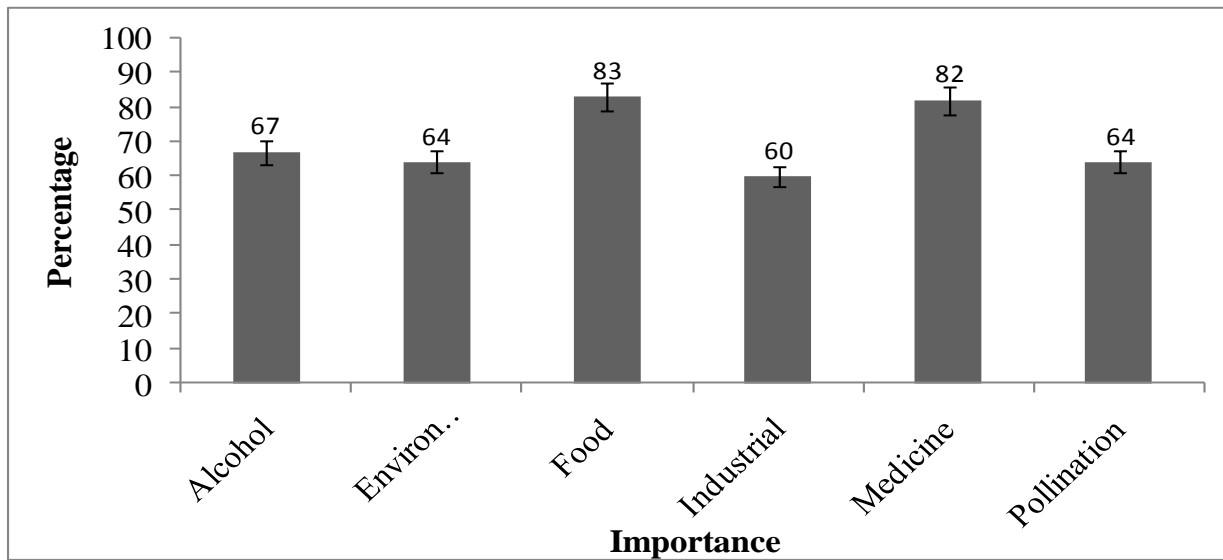
Figure 4.3. Importance of beekeeping enterprise in Transmara West Sub-County

These results confirm the farmers' appreciation of importance of honeybees. No wonder, no farmer sprayed pesticides to honeybees as target pests which is consistent with other findings.

Klein *et al* (2007) observed that around three-quarters of all global food crops, primarily vitamin-rich crops like fruits and vegetables depend on insect pollinators whose majority are insects such as bees. Kasina *et al* (2009) found that honeybees are the most commonly utilized for honey production besides pollinating cucurbits and sunflower. Furthermore, bee products were used as food, medicine and alcohol (Musimba *et al*., 2001).

Over 43 % of the respondents stated that their colonies were big, strong and high honey yielders in the past. About 16 % had weak colonies while14 % had low honey yielding colonies (Table 4.15). While 91 % observed change in their colony strength, 50 % observed a reduction in honey yields, 41% noted reduced colony size and 15 % observed weakening colonies.

Table 4.15. Colony strength dynamics in Transmara West Sub-County

Initial colony strength	Angata		Kilgoris		Lolgorian		Mean
	Frequency	%	Frequency	%	Frequency	%	%
Big and strong	59	66	42	49	40	42	43
Small and weak	6	7	30	35	16	17	16
High honey yielder	52	58	40	47	49	52	43
Low honey yielder	11	12	16	19	18	19	14
Colony change							
Yes	86	96	73	86	88	93	91
No	4	4	12	14	7	7	9
Observed changes							
Reduced size	43	48	27	32	42	44	41
Weakened colony	11	12	22	26	7	7	15
Lower honey yield	36	40	46	54	54	57	50
Causes of decline							
Pesticides	64	71	66	78	75	79	76
Deforestation	47	49	50	59	58	61	54
Drought	14	15	40	47	22	23	28
Pests and predators	29	31	20	24	6	6	20

n = 330

The survey revealed a declining colony population and production. This was attributed mainly to pesticides. Others possible causes were deforestation, drought, pests and predators. The results are consistent with past findings. For instance Musimba *et al.*, (2001) concluded that honey production in ASAL areas of Kenya has declined than in the past. Melathopoulos *et al.*, (2000), observed that honeybees are reportedly susceptible to pests, diseases and pesticides, which cause serious negative economic consequence to both the beekeeping industry and agriculture. In addition, it reaffirms Claudianos *et al.*, (2006) findings that honeybees are susceptible to pesticides due to a deficiency in the number of genes encoding for detoxifying enzymes, such as cytochrome P450 monooxygenases (P450s), glutathione-S-transferases, and carboxylesterases in their genome. Claudianos *et al.*, (2006), further observed that the relative lack of detoxicative genes in the honeybee genome further reduces the chances of a detoxicative gene response following pesticide exposure.

The survey revealed that 10 %, 30 %, 36 % and 25 % had sited their apiaries 10, 20, 50, and 100 metres away in that order from their spray crushes (Table 4.16). Majority of the beekeepers (88 %) believed that pesticides exposure had negative effects on honeybee, while 12 % stated that they had no effect on honeybees. The perceptions of the beekeepers regarding pesticides effects on honeybees were varied. Some 54 % believed pesticides caused outright bees kill, 30 % stated that they lead to reduced honey yields, 25 % mentioned hive absconding while 24 % stated that pesticides exposure to honeybees resulted in reduced colony size.

Table 4.16. Distance of pesticides use and effect on honeybees in Transmara West Sub-county

Distance between apiary and spray crush	Angata		Kilgoris		Lolgorian		Mean
	Frequency	%	Frequency	%	Frequency	%	%
10 metres	4	4	6	7	17	18	10
20metres	32	36	35	41	14	15	30
50metres	34	38	37	44	25	26	36
100 metres	22	24	7	8	39	41	25
Do pesticides affect bees							
Yes	73	81	78	92	86	91	88
No	17	19	7	8	9	9	12
Effects of pesticides							
Kill bees	56	62	34	40	55	37	54
Cause absconding	21	23	13	15	33	35	25
Reduce colony size	23	26	30	35	13	14	24
Reduce honey yields	26	29	27	32	28	30	30

$n = 330$

Most farmers sited their apiaries further away from the spray crushes. This was due to their belief that pesticides exposure to honeybees is determined by proximity; distance between cattle spray race and the apiary. It is expected that colonies close to areas being applied pesticides will severely be damaged due to intense exposure than those far away (Garcia *et al*., 1996). This observation is consistent with Henry *et al*., (2012) and Teeters *et al*., (2012) findings. They found that colonies located near treated crops where most of their workers are exposed to pesticides in nectar throughout cropping seasons could experience serious population decline.

Understandably, most farmers were reluctant to abandon pesticides use; this was despite their negative impacts on honeybees. Majority of them (74 %) recommended that pesticides that severely affected honeybees should not be banned but instead be reformulated while a lower proportion of the beekeepers, 26 % recommended banning of pesticides. 79 % recommended appropriate use of pesticides, manual tick control (14 %) while seven percent stated that ethno

herbs should be used instead to control pests and parasites (Table 4.17). The pesticides recommended for banning were the ones thought to be severely affecting honeybees. However pesticides remain important in control of pests in livestock and crop production in the study area.

Table 4.17. Mitigation measures of pesticides use effects on bees in Transmara West Sub-County

Mitigation measures	Angata		Kilgoris		Lolgorian		Mean
	Frequency	%	Frequency	%	Frequency	%	%
Ban pesticides	31	34	22	26	18	19	26
Reformulate	59	66	63	74	77	81	74
Suggested control method of parasites							
Manual tick control	25	28	5	6	8	9	14
Appropriate pesticides use	58	64	73	86	81	85	79
Use ethno herbs	7	8	7	8	6	6	7

n = 330

Hamilton and Crossley (2004) concluded that great productivity gains are achievable in agriculture by using adequate pesticides and are indispensable in meeting the global demand on food. According to Maya *et al*., (2012), the inappropriate pesticides use and subsequent accumulation in water, soils and air is detrimental to the environment. While this does not imply that pesticides are bad it acknowledges that their continuous inappropriate use tends to impact negatively on the environment (Muli *et al*., 2014). Therefore there is need for a balancing act so that pests are controlled while conserving biological diversity. One of the ways to achieve this is through labelling pesticides containers with environmental hazards in bold type and of conspicuous prominence (Williamson *et al*., 2008).

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

The study found that pesticides use significantly increased honeybee mortality rates ($p = 0.01$) although it was below the maximum station weekly threshold (250 dead bees). The mean mortality rate in treated colonies was 232 ± 4.6 while that in control and on-farm colonies were 73.0 ± 12.0 and 77.0 ± 5.9 respectively. The increase in mortality rate was found to result in a decrease in honey yields. The mean honey yield in control colonies was 20.30 ± 1.3 Kg while the on-farm and treated colonies yields were 14.22 ± 1.93 Kg and 10.6 ± 1.43 Kg respectively. No residues were detected in all matrices in both seasons suggesting that honeybee products in Transmara West Sub-County were safe for human consumption. The study further found that large proportion of farmers (91%) used pesticides; pyrethroids (50%), formamidines (25%) and organophosphorous (25%) classes to control ticks, tsetse flies, stem borer, aphids and beanflies. Although largely registered (95%), pesticides were haphazardly used without regard to safety measures. For example most farmers (79%) stored pesticides in granaries and applied them weekly (79%) during morning hours (93%) with 66% applying pesticides cocktails for efficacy purposes. About 83% disposed pesticides containers improperly.

5.2 Conclusions

The study found that pesticides use increased honeybee mortality rate significantly. This in turn resulted in a decline in honey yields.

There were no pesticides residues detected in all matrices across the study sites. The absence of residues in the matrices in all the sites and across Kenya, particularly when compared to levels in developed countries, suggests honeybee products are safe for human consumption in Kenya.

The classes mostly used were Pyrethroids, organophosphorous and formamidines duly registered by PCBP although majority of them are classified as moderately hazardous. There is inadequate information among farmers on pesticides handling particularly on; selectivity, dosages, time of application, storage and disposal. Livestock and crops were sprayed weekly during morning hours regardless of crops' physiological maturity including all flowering stages. Therefore the study concludes that there is inappropriate pesticides use in the study area.

5.3 Recommendations

1. Farmers should be encouraged to spray their livestock weekly.

2. Application of pesticides to crop fields should be done appropriately with frequency determined by the physiological maturity of crops and likely time of pests' emergence.

3. Extension services should be strengthened to ensure farmers handle pesticides safely

4. Pesticides containers must be properly labeled including hazard signs

5. The regulatory body; PCPB should conduct routine surveillance to ensure that only qualified personnel handle and dispense pesticides conditionally to farmers.

6. Highly hazardous pesticides should be abandoned and integrated pests management (IPM) Practices adopted

7. Used pesticides containers or expired pesticides should be buried or disposed in pits.

5.4 Suggestion for further study

Since this study focused only on pesticides use patterns and effect on honeybee's mortality and production, further research to compare pesticides effects in combination with other environmental stressors in the study area is highly recommended. This is because the combinations of these stressors seem to weaken honeybee colonies. Probably this may be increasing honeybee mortality.

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Appendix 1: Pesticides use in Transmara West Sub-county Questionnaire

Pesticides use by farmers to Control Crop and Livestock pests in Transmara West Sub-county

This survey is being carried out to determine the pesticides use patterns among farmers in Transmara and to assess the extent of damage caused by pesticide use to beekeeping industry in the Sub-county in order to make recommendations to the local community and policy makers on judicious use of pesticides. The information provided will be treated confidentially and no identities will be revealed.

Start time…………………..

Questionnaire No ………………………..Date of interview………………………………

Section A: **Site description**

1. County…………….………………Sub-county……………………………………….

   Division………………………………Location…………………………………..

   Su-location…………………………...Village………………………………………

2. Geographical positioning systems (GPS) readings

   Altitude…………………Latitude…………………… Longitude………………

Section B: Details of respondent

3. Name of respondent (farmer)………………………………Telephone No…………………

4. Relationship of respondent to the household head

   Self [1] Spouse [2] Son [3] Daughter [4] Others [5] specify ……………………………..

5. Gender

   Male [1] Female [2]

6. Age of respondent (farmer)

   Less than 18 [1] 18-35Years [2] 35-50 years [3] Over 50 years [4]

7. Highest level of formal education

   Illiterate [1] Non- formal education [2] Primary [3] Secondary [4] Tertiary [5] university [6]

8. Occupation
   Crop farming [1] Animal farming [2] Trade [3] Non-formal [4] formal employment [5]

Section C: Livestock

9. Which livestock do you keep and the approximate number?

    Cattle [1] =   [   ]   Goats [2] =   [   ]   Sheep [3] = [   ]

    Chicken [4] = [   ]   Donkeys [5] = [   ]   Pigs [6] =   [   ]

    Others [7] Specify…………………………………….

10. How is the mobility of your livestock?

    Sedentary [1] Nomadic [2] Others [3] Specify………………………

11. How do you graze your livestock?

    Herded [1] Paddock [2] Tethered [3] Free grazing [4] Others [5] Specify…………………

12. Which pests and parasites affect your livestock?

    Ticks [1] Mites [2] Tsetse flies [3] Lice [4] Worms [5]

13. How do you control ticks, mites and tsetse flies?

    Pesticides [1] Ethno drugs [2] Manual tick picking [3] Burning dry pasture [4]

14. Is there a cattle dip in your neighbourhood? Yes [1] No [2]

15. If yes, do you take your livestock to the cattle dip for parasites control? Yes [1] No [2]

16. If No, which pesticides do you normally use at home to control parasites? Fill the table below

Table 1: Pesticides used to control livestock parasites (call for pesticide containers)

Pesticide Trade Name	Common Name	Class	Volume (Lts)	Active ingredient	% of active ingredient

Classes: Insecticides (organochlorines, organophosphates, carbamates), acaricides and fungicides

17. Who introduced you to pesticides use?

    Neighbors [1] Friends [2] Agro dealers [3] Extension staff [4] Radio [5]

18. How do you learn of new pesticide products in the market?

Local leaders [1] MoA [2] Church [3] Media [4] Others [5] Specify………………………

19. Whom do you trust to be credible to give you information on pesticide products?

Community leaders [1] Leading farmers [2] NGOs [3] MoA [4] Media [5]

20. How often do you apply pesticides to your Livestock?

Weekly [1] Biweekly [2] Monthly [3] Bimonthly [4]

21. How do you apply the pesticides to your Livestock?

Hand sprayer [1] Knap sack sprayer [2] Hand dressing [3] Pour-on [4] Others [5] Specify……………………………

22. What time of the day do you apply pesticides to your livestock?

6-8am [1] 8-10am [2] 10-12am [3] Afternoon [4] evening [5]

23. How do you prepare the pesticide solution from the stock pesticide solution?
………………………………………………………………………………………
………………………………………………………………………………………
24. How long have you been using pesticides for Livestock parasites control?

Five years [1] Ten years [2] Fifteen years [3] Twenty years [4] Thirty years [5]

Section D: Crops
25. Do you practice crop farming? Yes [1] No [2]

26. If yes which crops do you grow? Fill the table below

Table 2: Crops grown and respective acreage

Crops	2013		2014	
	Plot size (Acres)	**Yields (Kg)**	**Plot size (Acres)**	**Yields (Kg)**
Maize				
Beans				
Sukuma wiki				
Cabbage				
Tomatoes				
Peas				
Sugarcane				

27. Which pests do affect your crops?

Stem borer [1] Aphids [2] Nematodes [3] Bean flies [4] Army worms [5]

28. How do you control the pests affecting your crops?

Pesticides [1] Manual [2] Do not control [3] Others [4] Specify………………………….

29. If pesticides, which ones and their active ingredients

Table 3: Pesticides used to control Crop pests

Pesticide Trade Name	Common Name	Class	Volume (Lts)	Active ingredient

Classes: Insecticides (organochlorines, organophosphates, carbamates and neonicotinoids), acaricides, fungicides and herbicides

30. Do you use one pesticide or a combination of more than one in a season/ time?
One [1] More than one [2]

31. If more than one pesticide, why?

Synergy [1] Safety [2] Others [3] Specify………………………………..

32. Who supplies the pesticides?

Agro dealer [1] GoK [2] Kiosks [3] Middle men [4]

33. How often do you apply pesticides to your crops?

Weekly [1] Biweekly [2] Monthly [3] Bimonthly [4] Others [5] Specify……………………

34. How do you apply the pesticides to your crops?

Hand sprayer [1] Knap sack sprayer [2] Hand dressing [3] Pour-on [4]

35. What time of the day do you spray pesticides to your livestock or crops?

6-8am [1] 8-10am [2] 10-12am [3] Afternoon [4] evening [5]

36. What physiological stage do you apply pesticides to your crops?

After weeding [ ] Flower Budding [ ] Flower Bloom [ ] Petal fall [ ]

37. How do you prepare the pesticide solution from the stock pesticide solution?

…………………………………………………………………………………………………….

…………………………………………………………………………………………………….

38. What volume of pesticide solution do you use per spray session (complete spraying of crops)

10 litres [1] 20 litres [2] 40 litres [3] 60 litres [4] 100 litre [5] 200 lites [6] Others [7] ……..

Section E: Quality Control and Safety

39. Do you have any pesticides in your store currently? Yes [1] No [2]

40. If yes fill the details in the table below

Table 4: Pesticides quality control and safety

Pesticide Trade Name	Active ingredient	Registrant	Registration No	Manufacturing date	Expiry date

41. Do you check the shelf life of pesticide products that you buy in the market?

Yes [1]   No [2]

42. If yes, what is the motivation?

Safety [1] Efficacy [2] Costs [3] Others [4] Specify……………………………………

43. Do you put on protective clothing (gloves, masks, overcoats and gumboots?) Yes [1] No [2]

44. Where do you store the pesticides?

Granary [1] Pesticides store [2] Living room [3] Others [4] specify…………………..

45. How do you dispose of the used or expired chemicals and empty containers?

Pit latrine [1] Rubbish pit [2] pouring on the ground [3] Others [4] Specify…………………..

46. Have you undergone any basic training on pesticide handling Yes [ ] No [ ]

47. If yes, who offered the training?

MoA [ ] MoH [ ] Agro dealers [ ] NEMA [ ] Environmental lobbies [ ]

Section F: Beekeeping

48. Do you keep honey bees? Yes [1] No [2]

49. If yes, how many hives do you own? Indicate in the table below

Table 5: Beekeeping practices

Hive	No of hives	No of hives colonized	Yields in 2014 (Kg)		Yields in 2015 (Kg)	
			Season 1 Jan-June	Season 2 July-Dec	Season 1 Jan-June	Season 1 July-Dec
Loghive						
KTBH						
Langstroth						
Total						

50. In your opinion do you think honey bees are of any economic importance? Yes [1] No [2]

51. If yes how?, mark as appropriate

Honey is food [1] Bee products have medicine properties [2] Honey is brewed to produce alcohol [3] Bees pollinate crops [4] Environmental conservation [5] Industrial benefits [6]

61. How long have you been keeping bees?

1-5 years [1] 5-10 years [2] 11-20 years [3] More than 20 years [4] Others [5]

62. How was the size and strength of your colonies when you started keeping bees?

Big and strong [1] Small and weak [2] High honey yielding [3] Low honey yielding [4]

63. Have you observed any changes in the recent past regarding your colony size and strength?

Yes [1] No [2]

64. If yes, how have been the changes?

Reduced colony size [1] weakened colony [2] Reduced honey yields [3]

65. What can be attributed to these negative changes? (Mark as appropriate)

Pesticide use [1] deforestation [2] drought [3] pests/predators [4] Diseases [5] Don`t know [6]

66. How far is your apiary from your spray race?

Ten metres [1] Twenty metres [2] Fifty metres [3] hundred metres [4] > 100 metres[5]

67. In your opinion, does the use of pesticides affect honey bees? Yes [1] No [2]

68. If yes above, how do they affect?

Kill bees [1] Cause absconding [2] reduces colony population [3] reduced honey yields [4]

69. Which pesticides affect honey bees the most? Rank in order of their severity

i..……………ii………………iii………………iv………………v…………………

70. What do you think should be done with the pesticides that affect honey bees the most?

Ban [1] Reformulate [2] Others [3] Specify……………………………….

71. Have honey bees ever caused you any inconveniences? Yes [ ] No [ ]

72. If yes how?

Stinging animals [1] Stinging humans [2] Quarrels with neighbors [3] Nuisance [4]

73. Have you ever sprayed honey bees with pesticides as a target? Yes [1] No [2]

74. In your opinion, what needs to be done to ensure that the honey bees are not adversely affected by pesticides?

Manual tick control [1] use pesticides appropriately [2] use ethno herbs [3]

75. How do agro dealers (manufacturers and distributors) respond to complaints?

Sensitization in Local barazas [1] Investigate Complaints [2] Product reformulation [3]

Change of pesticide [4] Take no action [5]

End time…………………………………………………………..

I wish to sincerely thank you for sparing your time to answer my questions. It is highly appreciated

Appendix 2: Krejcie and Morgan's Table for Determining Sample Size from a Given Population (1970)

N	S	N	S	N	S
10	10	220	140	1200	291
15	14	230	144	1300	297
20	19	240	148	1400	302
25	24	250	152	1500	306
30	28	260	155	1600	310
35	32	270	159	1700	313
40	36	280	162	1800	317
45	40	290	165	1900	320
50	44	300	169	2000	322
55	48	320	175	2200	327
60	52	340	181	2400	331
65	56	360	186	2600	335
70	59	380	191	2800	338
75	63	400	196	3000	341
80	66	420	201	3500	346
85	70	440	205	4000	351
90	73	460	210	4500	354
95	76	480	214	5000	357
100	80	500	217	6000	361
110	86	550	226	7000	364
120	92	600	234	8000	367
130	97	650	242	9000	368
140	103	700	248	10000	370
150	108	750	254	15000	375
160	113	800	260	20000	377
170	118	850	265	30000	379
180	123	900	269	40000	380
190	127	950	274	50000	381
200	132	1000	278	75000	382
210	136	1100	285	100000	384

Note: N= Population size, S= Sample size

Appendix 3: Drop-zone dead-bee under basket trap for monitoring bee mortality (Accorti *et al*, 1991)
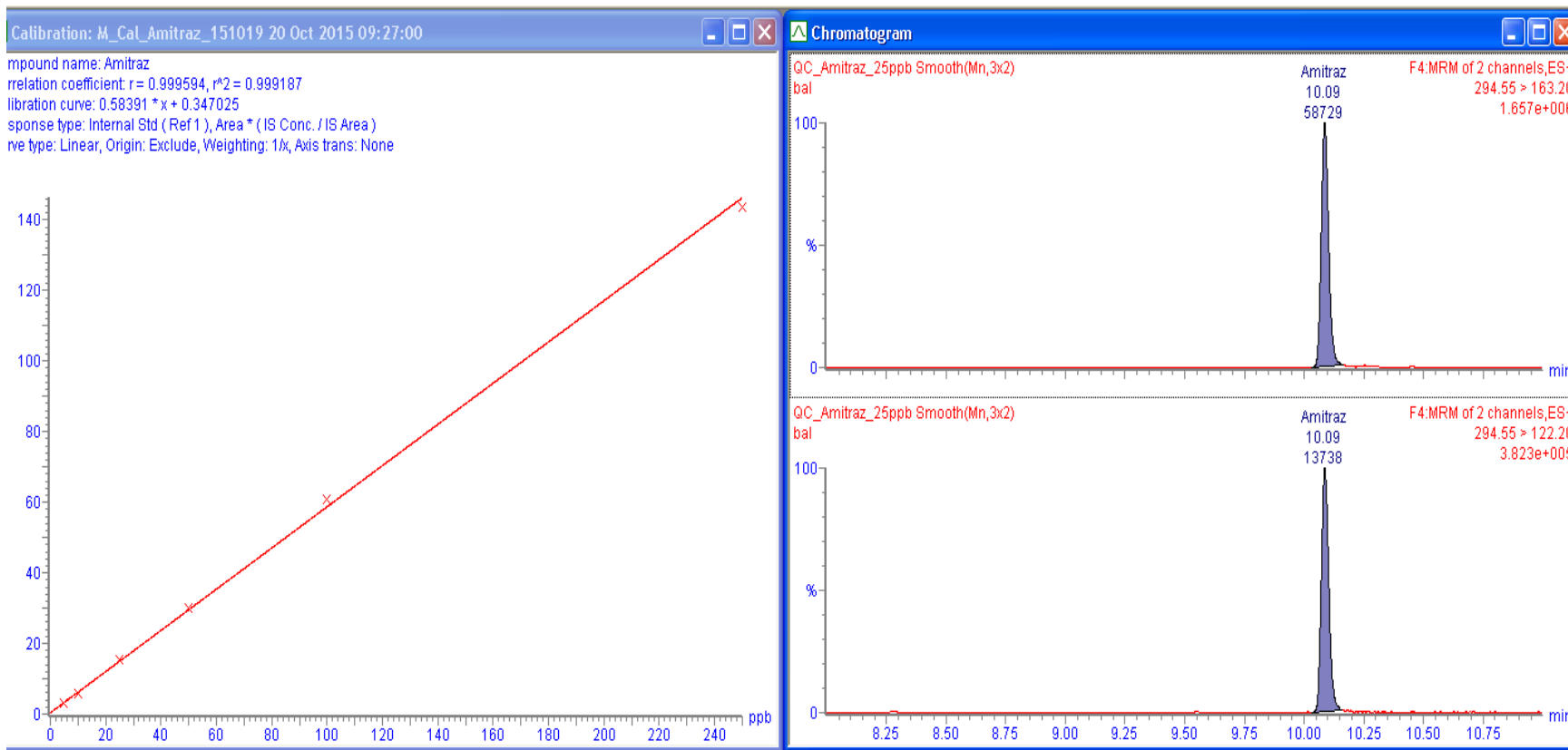
Appendix 4: The Researcher fixing dead bee traps to hives containing the experimental colonies
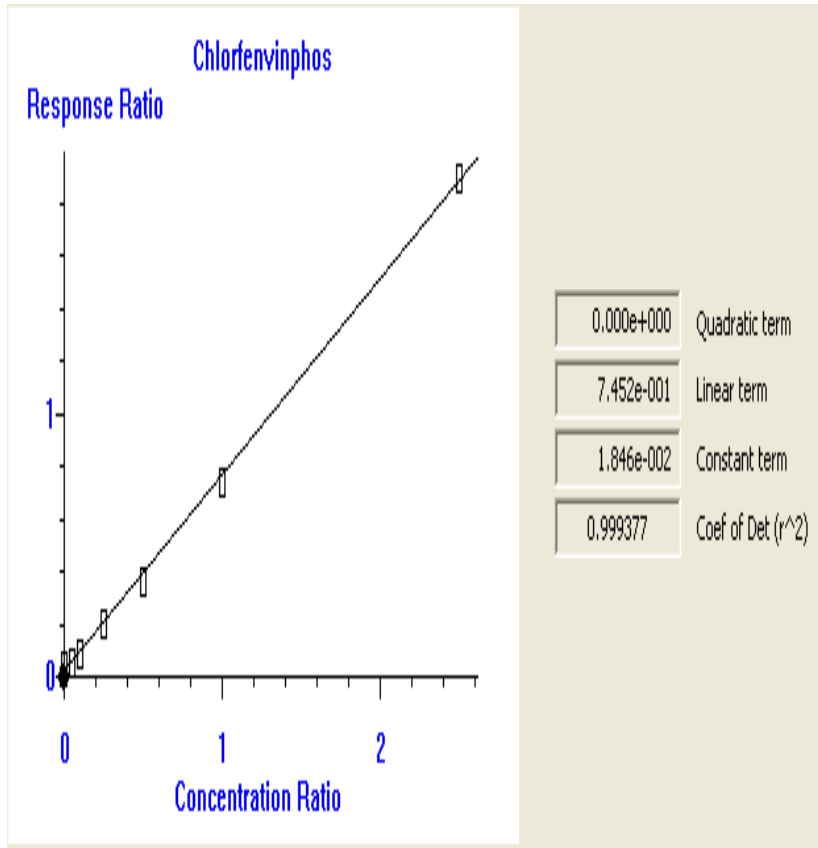
Appendix 5: Calibration curve and chromatogram for amitraz standard solution (25 ppb Spike and Recovery: 90%)
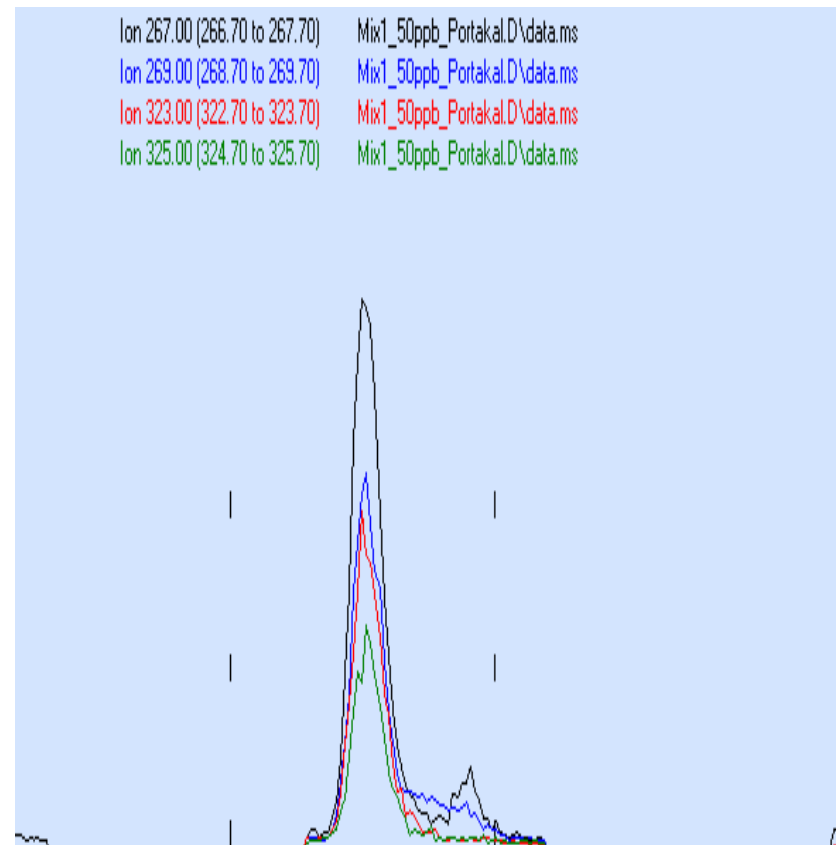

Calibration curve

25 ppb Spike and Recovery: 90%

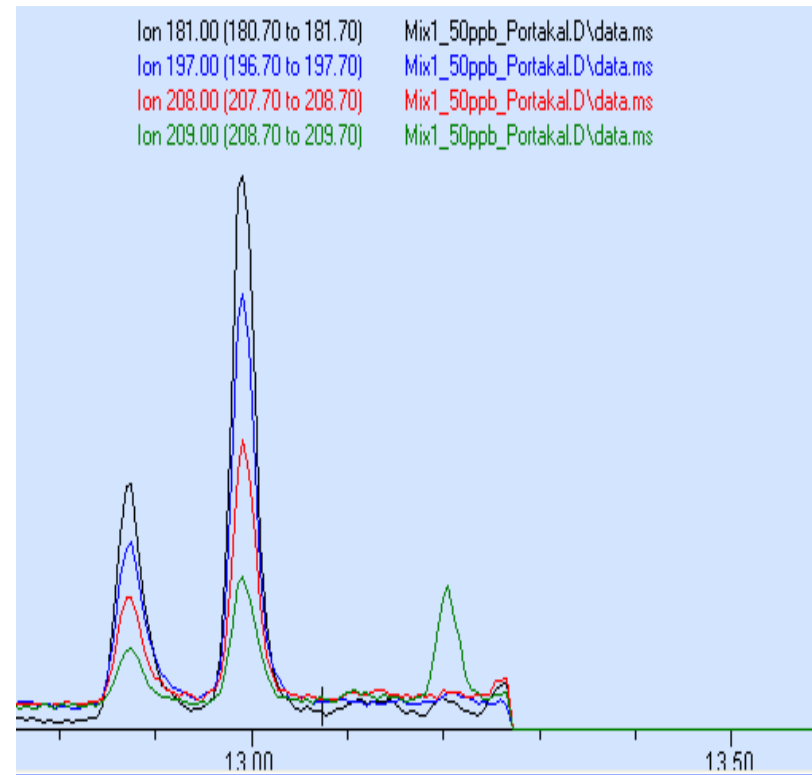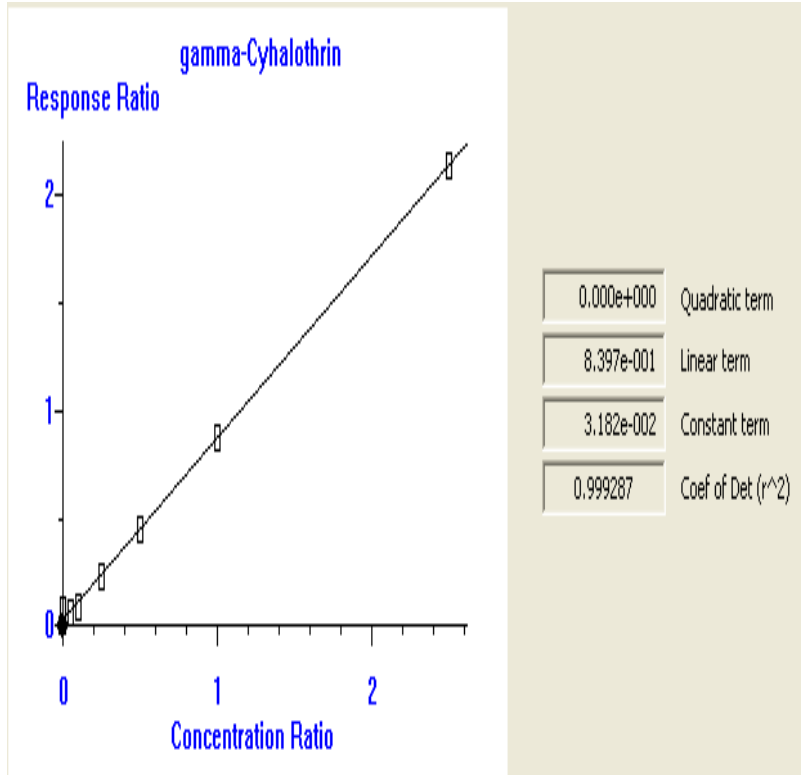Appendix 6: Calibration curve and chromatogram for chlorfenvinphos standard solution (50 ppb Spike; Recovery: 94%)

0.000e+000	Quadratic term
7.452e-001	Linear term
1.846e-002	Constant term
0.999377	Coef of Det (r^2)

Ion 267.00 (266.70 to 267.70) Mix1_50ppb_Portakal.D\data.ms
Ion 269.00 (268.70 to 269.70) Mix1_50ppb_Portakal.D\data.ms
Ion 323.00 (322.70 to 323.70) Mix1_50ppb_Portakal.D\data.ms
Ion 325.00 (324.70 to 325.70) Mix1_50ppb_Portakal.D\data.ms
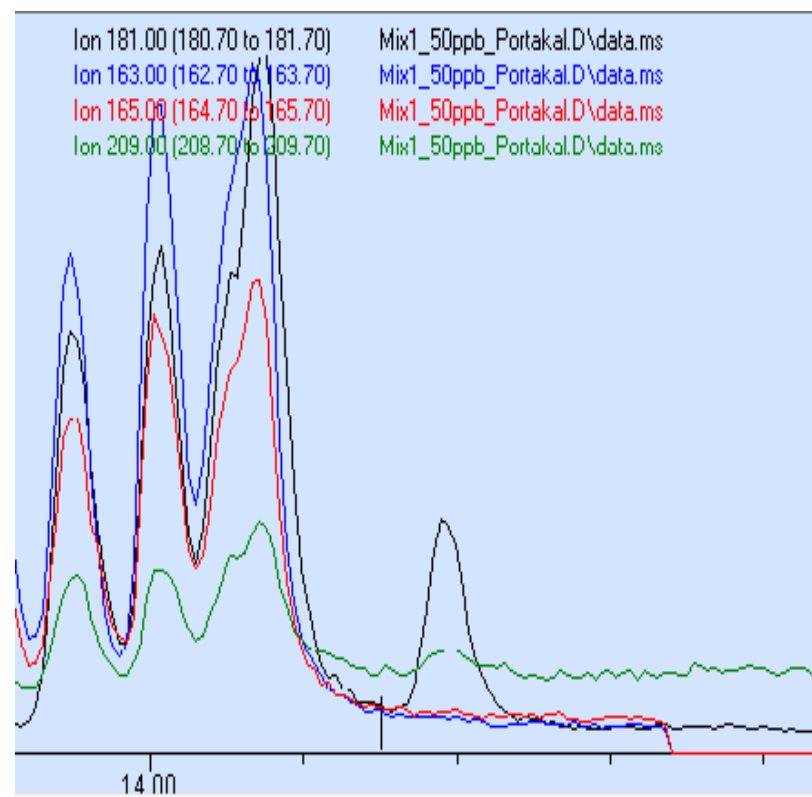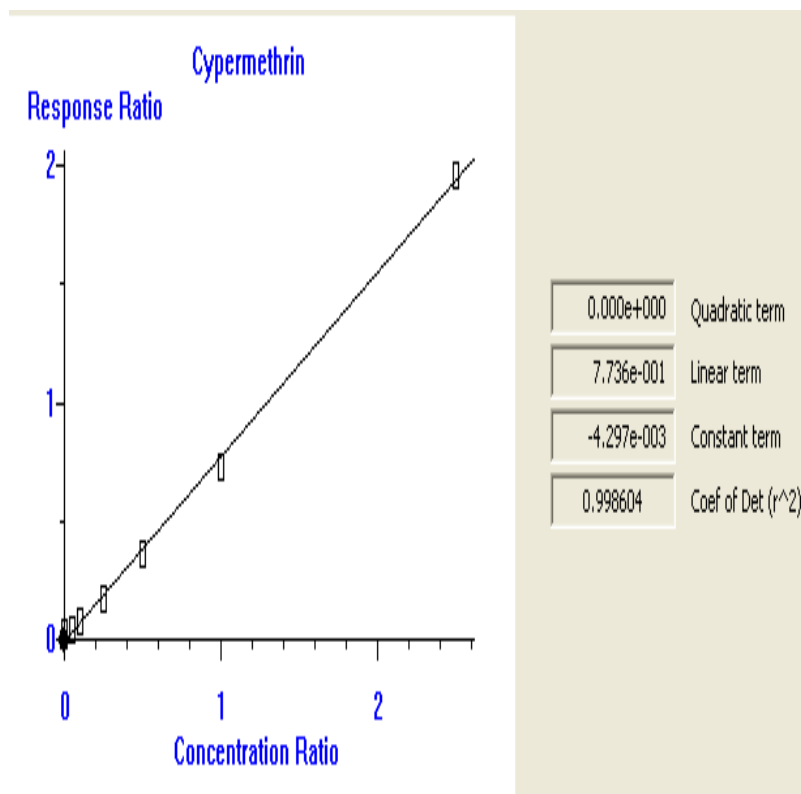
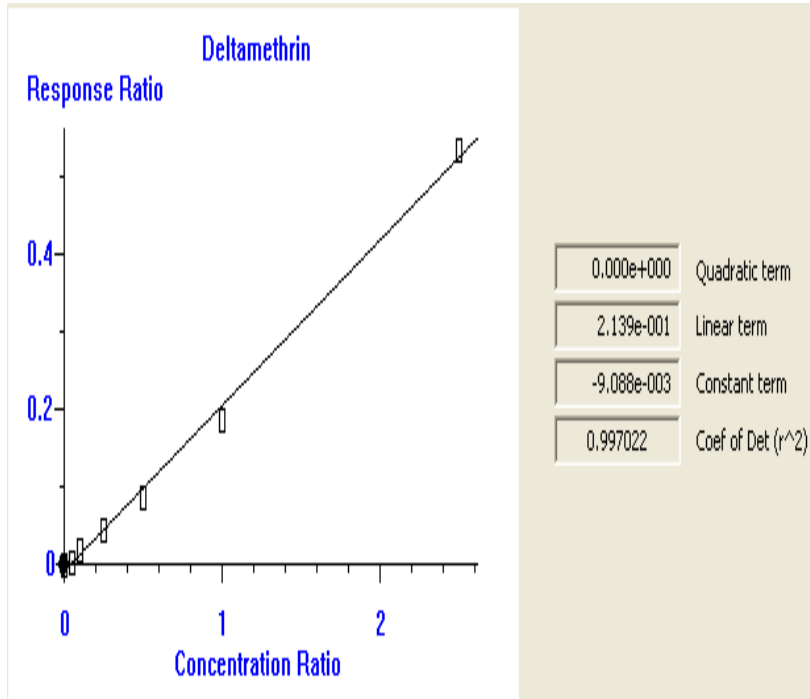**Calibration Curve**

50 ppb Spike; Recovery: 94%

Appendix 7: Calibration curve and chromatogram for Cyhalothrin standard solution (50 ppb Spike; Recovery: 97%)

Appendix 8: Calibration curve and chromatogram for cypermethrin standard solution (50 ppb Spike; Recovery: 92%)

0.000e+000	Quadratic term
7.736e-001	Linear term
-4.297e-003	Constant term
0.998604	Coef of Det (r^2)

Ion 181.00 (180.70 to 181.70)   Mix1_50ppb_Portakal.D\data.ms
Ion 163.00 (162.70 to 163.70)   Mix1_50ppb_Portakal.D\data.ms
Ion 165.00 (164.70 to 165.70)   Mix1_50ppb_Portakal.D\data.ms
Ion 209.00 (208.70 to 209.70)   Mix1_50ppb_Portakal.D\data.ms

Appendix 9: Calibration curve and chromatogram for Deltamethrin standard solution (50 ppb Spike; Recovery: 93%)

0.000e+000	Quadratic term
2.139e-001	Linear term
-9.088e-003	Constant term
0.997022	Coef of Det (r^2)

Ion 181.00 (180.70 to 181.70): Mix1_ElmaSuyu_50ppbSpike.D\data.ms
Ion 253.00 (252.70 to 253.70): Mix1_ElmaSuyu_50ppbSpike.D\data.ms
Ion 255.00 (254.70 to 255.70): Mix1_ElmaSuyu_50ppbSpike.D\data.ms
Ion 251.00 (250.70 to 251.70): Mix1_ElmaSuyu_50ppbSpike.D\data.ms
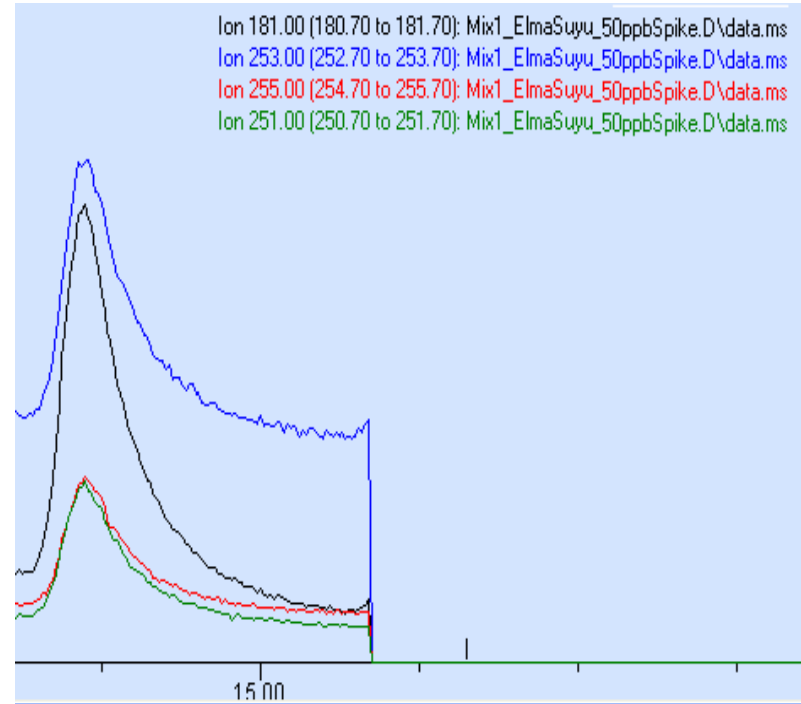
Calibration Curve   **50 ppb Spike; Recovery: 93%**

Appendix 10: Calibration curve and chromatogram for Dietathyl-ethyl (internal standard solution)