COLLEGE OF SCIENCE AND TECHNOLOGY

SCHOOL OF SCIENCE

Assessment of Pesticide Residues in Honey from Kayonza District-Rwanda

A dissertation submitted to the Department of Chemistry, School of Science, College of Science and Technology, University of Rwanda, in partial fulfillment of the requirements for the Degree of Masters of Science in Environmental Chemistry.

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Kigali, August 2024

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DEDICATION

I dedicate this dissertation to my mother, who sponsored my education and has shaped an eager spirit in me to learn more. I further dedicate my dissertation to my consort who has been patient during the period of my study. Lastly, I dedicate this work to my sisters and brothers, who always give me the courage to make headway.

ACKNOWLEDGEMENT

Enough has been done throughout my education, thanks be to GOD the Almighty for granting me life and courage throughout my work.

Many people contributed to the completion of this work; I would like to express my sincere thanks to everyone who assisted and supported me to reach the completion of this work.

I would like to recognize all the staff of the University of Rwanda, especially my lecturers and classmates, for their generous and brilliant encouragement given to me to overcome some hard times during my studies. May the founder and other members of this institution find my cordial thanks for their work. I am indebted to my supervisors: Dr. Jean NTAGANDA (Ph.D.), and Dr. Jean Bernard NDAYAMBAJE(Ph.D.), who have been determinants to my academic research, without them, this work would not be possible. I appreciate and recognize your kind support for my work. May God Bless you!

Special thanks to the International Science Program (ISP) of Uppsala University, Sweden, for its continuous support of the Laboratory of Chemistry of the University of Rwanda in which this study was conducted.

Special thanks go to FIOM Rwanda and beekeepers in Kayonza District (Cooperative COPANYAKA, DUTUBURE UBUKI, COPROMA, IMPALA, ABATAVOGERWA, and Innocent NZABONIMPA) for their help in the honey sampling phase.

I owe my thanks to my family, wife, sister, brothers, and all my friends, who sacrificed their time, comfort, and support throughout this work and success, and all others with whom we shared the struggle for success. May God bless each, and every person mentioned and unmentioned for their support in the realization of this work.

ABSTRACT

Assessment of pesticide residues in honey from Kayonza District, RWANDA was conducted for six different pesticides: Abamectin, Profenofos, Alpha-cypermethrin, Chlorothalonil, deltamethrin, and Metalaxyl which are commonly used in Rwanda Easter Province, and their levels were evaluated under laboratory states. Pesticide residue in honey is one of the significant parameters to evaluate environmental contamination, and in this regard, twenty-eight (28) samples of fresh *Apis mellifera* honey were gathered from three different geographic areas (sector) namely Kabare, Kabarondo, and Ndego, in Kayonza District. After collection, these samples were transported in good condition by using a cool box and stored in the laboratory at a temperature between 4-10^{0}C until analysis. Extraction of pesticide residues in samples was carried out using water and ethyl acetate. High-Performance Liquid Chromatography (HPLC-UV) was used to identify and quantify the residues of these six distinct pesticides following the extraction and cleanup of honey. So far, results from laboratory analysis of pesticide residues have shown that 17% of honey samples are polluted by pesticide residues. Abamectin and deltamethrin pesticide residues were found, and the detection ranges were * to 0.048mg/kg for abamectin and * to 0.015 mg/kg for deltamethrin. Three samples of honey from the Ndego Sector contained traces of abamectin, while one sample each from the Ndego and Kabare sectors contained traces of deltamethrin. The range of residues detected is below the MRLs for Abamectin and Deltamethrin whose values are 0.05 and 0.03mg/kg, respectively, while profenofos, alpha-cypermethrin, metalaxyl, chlorothalonil residues were not found in all of the samples examined. According to the findings of this study, Honey from Rwanda is free from pesticide residues when results are compared to maximum residue limits but farmers and beekeepers must create a plan for their use of pesticides to avoid probable danger to health.

Keywords: *Extraction, HPLC, Pesticides, Limit of Detection, Maximum Residues Limits*

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List of acronyms/abbreviations

µg: Microgram

CCD: Colony Collapse Disorder

EC: Emulsifiable concentrate

EDPRS: Economic Development and Poverty Reduction Strategy in Rwanda

EU: European Union

FAO: Food and Agriculture Organization

FIOM RWANDA: Future In Our Minds Rwanda

GAP: Good Agricultural Practices

GC-MS: Gas Chromatography-Mass Spectrometry

HPLC-MS: High-Performance Liquid Chromatography Mass Spectrometry

IPM: Integrated Pest Management

ISO: International Standard Organization

LOQ: Limit of Quantification

LOD: Limit of Detection

MDGs: Millennium Development Goals

MRL: Maximum Residue Limits

NISR: National Institute of Statistics of Rwanda

PPM: Part Per Million

PPPs: Plant Protection Products

RICA: Rwanda Inspectorate Competition and Consumer Protection Authority

RPM: Revolutions Per Minute

RSD: Relative Standard Deviation

RS EAS: Rwanda Standard East Africa Standard

RT: Retention Time

RWANDA FDA: Rwanda Food and Drugs Authority

SAS: Seasonal Agricultural Survey

SDGs: Sustainable Development Goals

WP: Wettable Powder

CHAPTER 1: GENERAL INTRODUCTION

1.1. Research background

One of the naturally delicious substances made by honey bees from flower head nectar is called honey. It is a complex natural food and unquestionably the only sweetener that is unprocessed [1]. Since ancient times, it has been used as both a raw food and a medicinal herb. Basically, honey is a combination of different sugar products, particularly glucose and fructose [2].

Honey is frequently consumed by children, the elderly, and ill individuals, predominantly in developing nations. Therefore, for the safety reason of human feeding, honey has to be free from all pollutants. Although the overuse of pesticides has led to a number of ecological issues, one of which is pesticide residues in food, which pose a risk to human health [3], honey is subjected to various aspects, including the practice of insect killers, contamination, collecting practices, neighboring atmosphere, the well-being of honey bees and beehive sanitation [4].

In the past 50 years, agricultural practices have undergone chief changes from traditional to modern farming. The transformed farming practices have resulted in advanced harvests, yet there has also been a deterioration of different kinds of living organisms [5].

Organisms on the grounds and in nearby ecosystems have been impacted directly and indirectly by the increased application of pesticides in agricultural processes. However, using pesticides is significant for farming, because it safeguards crops from pests such as weeds, insects, and fungi their leftovers eventually end up in various environments and could harm the environment [6].

Honey bees, *Apis mellifera*, do the important work of fertilizing farming harvests and are significant in producing honey and beeswax. Between 10,000 and 25,000 honey bee workers perform about 10 tours a day to get to the places of interest, covering about 7km^2 in the zone around their beehive keeping, to collect pollen, water, and nectar from the lowers. Through this journey, chemical materials and a lot of microbes are taken by these honey bee workers and kept in their body surface hair [1].

1.2. Problem Statement

The use of pesticides in agriculture has substantially assisted the growth of agricultural productivity, however their supervision is an environmental, and public safety issue due to their high solubility in water, volatility and reaching air, and long shelf-life (persistence). Honey bee workers, in order to collect pollen, nectar, and water, interact with different materials where pesticides are applied and can transport pesticides in their honey hives [3]. There are not many studies tracking pesticide residues in honey products sourced from Africa, specifically East Africa. Over time, the accumulation of pesticide levels and their presence in honey products can cause health issues for both honey bees and honey consumers [7].

1.3. Objectives

1.3.1. General Objective

To investigate the levels of pesticide residues in honey from Kayonza District, Eastern Province is the primary objective of this study.

1.3.2. Specific objectives

The present research has specific objectives for:

- **(a)** To evaluate the levels of Abamectin, Profenofos, Alpha-cypermethrin, Deltamethrin, Metalaxyl and Chlorothalonil residues in honey samples from Kayonza District;
- **(b)** To compare the detected pesticide residue levels in honey with the MRLs set by regulatory bodies like the European Commission to assess if the honey is safe for consumption;
- **(c)** To provide recommendations to the local authorities and stakeholders on measures to ensure honey from Kayonza District remains free from pesticide contamination and meets international food safety standards.

1.4. Research questions

This study is subjected to the following questions:

- **(a)** What is the pesticide residue level in the honey of Eastern Province/Kayonza District?
- **(b)** Do the levels of pesticide residues detected in honey samples from Kayonza District, Rwanda exceed the MRLs set by the European Commission?
- **(c)** What measures can be implemented by local authorities and stakeholders in Kayonza District, Rwanda to ensure that honey production and processing remains compliant with international pesticide residue limits and food safety standards?

1.5. Significance of the study

Promoting quality culture in all sectors is one of the broad sector objectives of Rwanda's quality policy, and specifically producing high-quality honey for export and local consumption [8], [9]. Production of high-quality honey is an ambition of Rwanda because it is a chance to transfer honey outside to well-paid European Union (EU) marketplaces, and this gives approval to its residue monitoring plan and updating the growth of the honey industry [10]. As there is competition between honey traders, the latter must first be able to demonstrate that their honey is of good quality. According to the European Honey standard (Council Directive 2001/110/EC) and FAO Codex standard for honey, it must include only itself and cannot have any of its nutritional components removed [11]. Various pollutants can be brought into the hive by honey bees which visit different plants and get into contact with deposited pollutants. Crop safety products applied in agriculture can create toxic conditions for bees as well as bee products; specifically honey disturbing, properties and causing a particular risk to human well-being. Honey can serve as a sign of environmental toxicity with harmful compounds including pesticides [12]. Beekeepers have become more eco-conscious during the past few centuries that allow them to build their honey colonies successfully from a variety of blooming plants. As honey is a product for human feeding with highly valued by health specialists in the nutrition field, the importance of honey quality management stems from the fact that the environment has an important impact on the grade of its pollution with various poisonous impurities, and its pollution with several compounds has to be regularly studied [13].

1.6. Scope of the study

The scope of this study was constrained in both time and location; in accordance with the required work and available money, the study was carried out from March 2022 to March 2023 in one District of Rwanda's Eastern Province. The following six pesticide: Abamectin, α-Cypermethrin, Profenofos, Deltamethrin, Chlorothalonil, and Metalaxyl were focused on in raw honey, but their metabolites were not taken into accounts. The research was conducted in the Kayonza District, one of the three Districts; Ngoma, Kayonza, and Gatsibo, where FIOM Rwanda is implementing a Pro-Bee Project ‒Project for the responsive beekeeping value chain in the Eastern Province of Rwanda‖, a bordering zone of the Akagera National Park, which is one of four designated zones of high probable in beekeeping in Rwanda due to their position (near the lakes and where several bee species are likely to live) and their content in numerous species of plants as these are the core features for bees to produce high quality and quantity of natural honey. And also, the buffer zone in Kayonza District on delimitation of the Akagera National Park has many benefits for beekeepers, who have the prospect of using it as additional revenue, while also ensuring the protection of the park's boundaries and lowering poaching [14]. The honey samples were taken at Ndego, Kabare, and Kabarondo Sectors in the Kayonza District, where apiculture farming is practiced. At Kayonza District, the study was focused on first, collecting data from farmers and beekeepers, and lastly, sampling honey from hives and transporting it in a good manner to a laboratory for analysis.

CHAPTER 2. LITERATURE REVIEW

2.1. Types of crops and pesticides in Eastern Province

The economy of Rwanda is agriculture-based, with about 90% of its population. Crop intensification has been identified because agriculture has been defined as an economic engine in Vision 2020 and the EDPRS. The national agricultural policy and strategy of agricultural transformation will be used to help achieve the aforementioned aim and a means of achieving the MDGs and SDGs and reducing poverty. The adoption of high-yielding seeds, more fertilizer, and pesticides were all part of the crop intensification process [15].

The Agricultural Household Survey (AHS) conducted by the National Institute for Statistics (NISR) in the 2020-2021 agricultural year provides information on the use of pesticides during seasons A, B, and C in Eastern Province. Surveyed pesticides were Dithane M45/Mancozeb, Dimethoate, Cypermethrin, Ridomil, Dursban, and Rocket, and others. In the Eastern Province, the types of pesticides commonly used are given in the following Table 1 but not limited to those only.

Table 0-1: Type of pesticides, per Season and in Eastern Province in 2021 (%)

Season / **Pesticide** / **District**	**Season A**				**Season B**				**Season C**			
	Dithane-M	Ridomil	Cypermethrin	Rocket	Dithane-M	Ridomil	Cypermethrin	Rocket	Dithane-M	Ridomil	Cypermethrin	Rocket
NYAGATARE	6.9	1.3	11.1	30.5	15.8	2.8	9.5	28.4	26.6	-	11.7	20.8
GATSIBO	13.1	2.0	6.6	54.5	20.6	-	5.6	56.9	34.1	2.3	6.8	12.8
KAYONZA	17.9	2.2	9.4	24.1	23.3	2.1	11.0	27.4	36.7	1.8	5.5	34.1
NGOMA	10.0	-	11.3	60.0	23.0	1.2	23.0	27.6	29.0	1.9	5.5	30.8
KIREHE	6.7	1.8	17.6	47.3	18.3	4.8	19.1	20.6	22.0	1.7	13.6	35.6

Source: NISR, SAS 2021

The major crops are maize, beans, sorghum, soya beans, cassava, Chilies, ground nuts, sunflower, others plants are eucalyptus, grevillea, acacia, and some vegetables (Carottes, eggplants, cabbages, tomatoes) [14]. From a survey, interviewed farmers and Agro-dealers have responded that the most used pesticides in this zone are Kiyoda (Cypermethrin 5EC), Chlorothalonil, Rocket (Cypermethrin 4% + Profenofos 40%) EC, Rapid (foliar fertilizer), Lamdex (Lambda Cyhalothrin 2.5 % EC), Dethane (Mancozeb 80 % WP), Dudu (abamectin 1.8% EC or Acelactin 5% EC), Metalaxyl, Deltamethrin 2.5% EC, Simikombe (diazinon), and Rava (Dichlorvos).

2.2. Pesticide uses in Rwanda

Rwanda still only uses pesticides on high-value crops like fruits, vegetables, potatoes, and coffee. In certain sections of the nation, the availability and price of these agrochemicals are major issues. The national average for pesticide application in 2019 was less than 1 kg/ha. The majority of pesticides used are fungicides (75%), while other insecticides and a small number of herbicides make up the remaining 25% [16]. The most often used fungicides and insecticides, respectively, are dithiocarbamates and pyrethroids (Figure 1 and Appendix I).

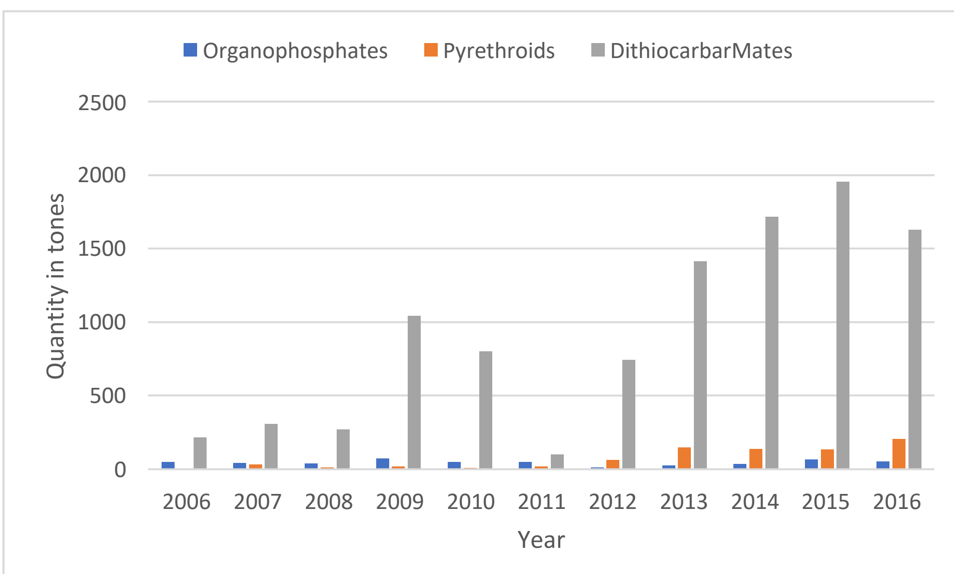

Figure 1: Variation of most applied pesticides in 15 years

Source: [16]

2.3. Honey pollutants

Honey can be polluted by various sources. The pollution can come up from the environment or apiculture practices. Heavy metals including lead, Cadmium, and Mercury, pesticides (insecticides, herbicides, fungicides, and bactericides), and pathogenic microorganisms are all examples of environmental pollutants. The contaminants from apiculture methods including products like organic acids, components of essentials oils, antibiotics for bee brood diseases, especially sulfonamides, and chloramphenicol [17].

2.3.1. Environmental pollutants

The use of pesticides in farming for crop defense and pest control has been linked to environmental pollution and anthropological health complications on a global basis [18] [19]. In Rwanda and bordering countries, pesticides are habitually used by resident growers without sufficient information on security procedures and suitable practices [3]. This leads to the application of large doses, consistent pesticide disposal, high amounts of pesticide byproducts in the air, environmental pollution through rainwater transportation, and soil leaching. Because local growers choose to purchase cheaper pesticides through illegal trade, it is common in Rwanda to see the use of prohibited pesticides. This causes Rwanda's water bodies to become contaminated with prohibited goods, exposing residents to pesticides through their drinking water [3].

Initially, pesticide concentrations can be high in the raw materials of honey, but the concentrations are reduced by about a factor of 1000, so the concentrations in honey are finally lower. Similarly, many modern pesticides are unstable and degrade quickly if applied [20].

2.3.2. Apiculture pollutants

Similar to all existing creatures, diseases and pests can infest honey bees. Some bee diseases are European (*Melissococcus plutonius*) and American foulbrood (*Paenibacillus larvae*), and acaricides are applied in apiculture to combat the small hive beetle (*Aethina tumida*), acariosis (*Acarapis woodi*), and varroasis(Varroa destructor). Nosemosis(*Nosema apis or N. ceranae*) and nosebleeds (*Aethina tumida*) can be treated with chemotherapeutics drugs and antibiotics respectively [20].

The *Varroa mite* is one of the honey bees' most hazardous parasites worldwide, also known as *Varroa destructor*. Beekeepers are usually required to apply varroacids in order to prevent honey bee association deaths because they harm both adult and young bees. Given that honey bees are sensitive to many pesticides, varroacids must be as gentle on the bees as possible while still being lethal to mites. The three main types of varroacids used are synthetic organic, natural products, and organic acid pesticides [21].

The conditions in honey do not permit the duplication, and even the persistence of microorganisms [17] [20]. But epidemiological data have demonstrated *Clostridium botulinum* and other clostridia producing botulinum neurotoxins to be the lone microbiological danger in honey. Even though *Bacillus spp*. are frequently identified in honey, sometimes in high numbers, there is no record of their having caused illness [22].

Although *Clostridium botulinum* reproductive structures can survive in honey, they cannot produce the poison. *Infant botulism* has occasionally been treated with honey in a few isolated cases. As a result, certain honey packers (The British Honey Importers and Packers Association for example) have been ordered to add additional material as a warning that ‒honey should not be given to babies below one year‖. On the other hand, this microbe is present in all-natural foods throughout the world [17] [20].

2.4. Exposure of bees to pesticides

High quantities of agricultural pesticides and in-hive varroacids are being exposed to honey bees. It is well known that honey bee fitness declines after repeated exposure to neurotoxic pesticides and their mixtures by means of further pesticides, particularly fungicides. However, the cause of this diminishing honey bee health is still unknown. Both environmental and beekeeper methods can contaminate honey (Figure 2) [23]. Pesticides used in farming can be expended into the bee products' basic materials by different routes, such as in air, water, plants, and soil, thereafter, the bees might carry them into the beehive [2]. Most pesticides are applied on farms by spraying over the entire produce, then sprays of weed killers, and usually, antifungal medications are applied straight on the soil prior to the planting of harvests. In these situations, sand and localized drops where pesticides are applied drop straight on the bees that hover crossways the treated grounds or neighboring fields as the wind can transport the tiny units hundreds of meters out of the farm [24].

Typically, pesticide residues contained in pollen and nectar of through the ingestion of residues contaminated plants expose bees to pesticides through eating, such as weeds around farms or crop plants [7]. It is more significant to note that bees circulate everywhere to find the most appropriate flowers that give honey bees food in high amounts. Therefore, some plants are more alluring to bees than others. Therefore, some plants are more attractive than others to bees. As a result, some crops, like flowers with a yellow color, are more beautiful than others (sunflower), and various wild plants that grow in and from place to place attract bees more than potato plant flowers. Pollen and nectar that contain pesticide residues are transferred to bee colonies and honey [25].

Besides pollen and nectar, food for honey bees, these last also drink water to preserve their organism's heat or coolness in normal conditions [26]. Pesticide residues, like other particles in the environmental compartments, move from one place to another (Annex 4: Pesticide Mobility in Environment), that's why they are found in soil and finally move to water in streams and farm pond zones and outside, which is then polluted by different kinds of pesticides [27]. Pesticides are exposed to in the environment in a variety of ways; honey bees are not exposed to just one or two chemicals but rather a mixture of numerous agricultural substances [7].

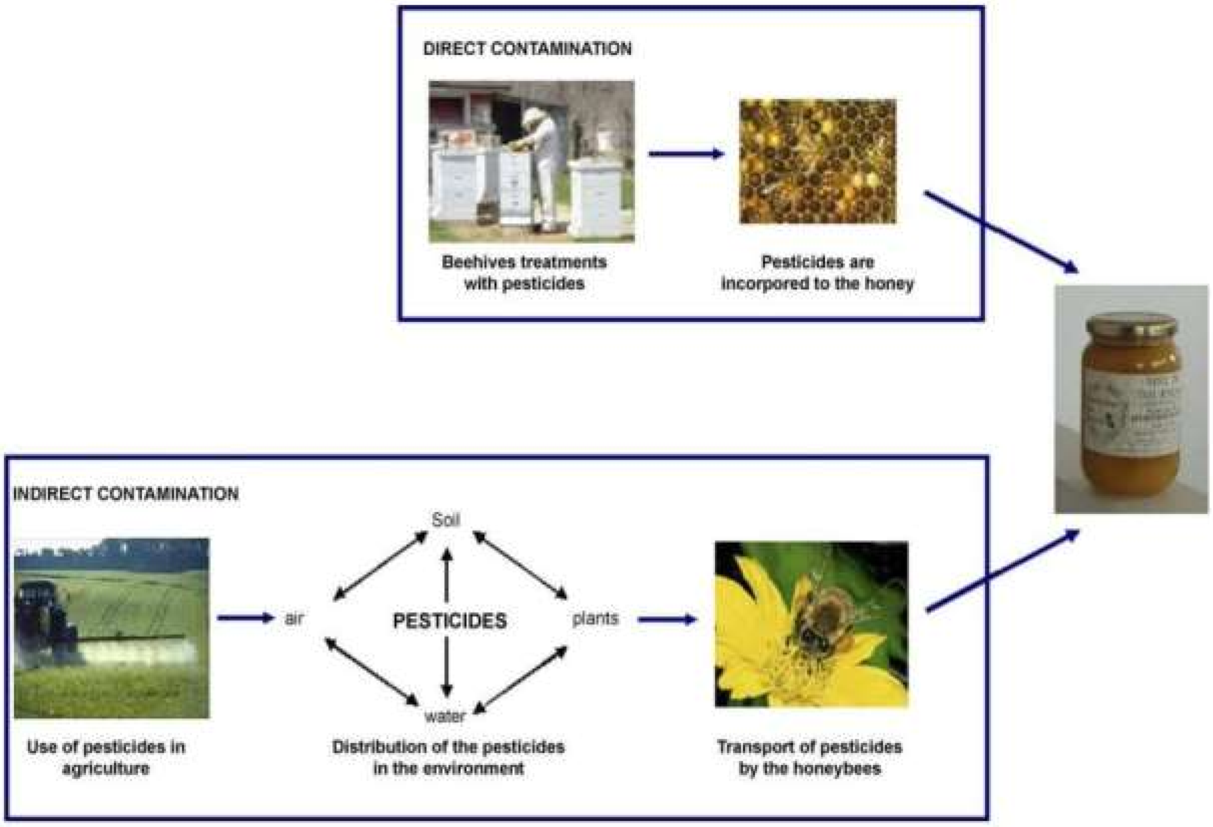

Figure 2: Routes of honey contamination with pesticides

Source: [28]

Honey bees are continually exposed to acaricides (Amitraz, Cymiazole, Flumethrin, Bromopropylate, Coumaphos, and Fluvalinate) in addition to pesticides used in agricultural crops [29] to manage parasites such as Varroa. In this instance, honey bees interact with high pesticide residue levels that are existing on the waxy comb cells, primarily impacting the larvae that are still developing [7].

2.5. Pesticide residues in honey and health Hazards

2.5.1. Health Hazards to humans

While pesticides are used to destroy pests, some of them can have detrimental effects on environment and human health as well. Acute and chronic poisoning can result from ingesting, inhaling, or coming into contact with pesticide residues on the skin. These toxicity levels are influenced by pesticide categories, quantity, entrance point, digestion, accumulation, and other factors [30]. The relations among the wellbeing of environmental service benefactors and anthropological wellbeing continue indefinitely. Through the ancient period, a cumulative various of researches have claimed the optimistic effects of healthy pollinator societies on anthropological health [31]. In the practice of protecting agricultural crops from pests and diseases, pesticides are applied to farms, and pesticide residues accumulate in foodstuffs, including honey bee products. Pesticide pollution in a confined environment can be replicated by pesticide residue in honey and other bee products. The pesticide residues in honey products threaten human health [32]. By using pesticides in agriculture and apiculture, there is an excessive yield from crops. Actually, the pesticides are necessary to meet the global standard requirement for a variety of food goods, and there isn't a replacement that can rival them on such a wide scale. When consumed through various food chains, farmers' irresponsible handling and sluggish decay could contaminate the environment and harm people [33].

The use of pesticides on crops on farms has caused complications even for off-target organisms, and leading to various pathologic diseases that interrupt the processes of biological functions. Anomalies in the central nervous system, haemangiomas, orofacial clefts (birth deformities), urogenital defects including hypospadias and cryptorchidism, circulatory/respiratory, gastrointestinal, and musculoskeletal pathologies, haematomas, and other diseases are the hazards of pesticide residues in relation to human contact [34].

Forager bees transport pesticides to honey bee hives as they gather nectar and pollen from various plants where those pesticides have been sprayed. Similarly, the application of pesticides in apiculture causes deposits of pesticide residues in honey since these different pesticides are sprayed inside the beehive to avoid and remove a certain number of infections [17].

2.5.2. Health hazards to honey bees

Residues of pesticides developed by honey bee foragers are correspondingly greater in pollen (kept and surrounded at the beehive entry), mature honey bees, and rarely in honey than in beeswax [35].

Pesticide exposure with pyrethroid, organochlorine, organophosphorus, and carbamates was responsible for the majority of honey bee deaths between 1966 and 1979. More efforts were put in place to restrict pesticide usage during flowering, but the residual action of some insect killers was not efficiently directed. Assessments and publications for the safety of honey bees regarding the different classes of pesticides are accessible [35].

Varroa destructor (1987) and *Acarapis woodi* (1984), two parasitic honey bee ticks, appeared later. At the same time, genetic Engineering (GE) was quickly changing how crop pests were controlled in USA agricultural crops, and the two innovative groups of systemic insecticides; neonicotinoids and phenylpyrazoles, substituted several of the ancient insecticides. Among pesticides, neonicotinoid insecticides are the major hazard. Due to their widespread usage, pesticides can be identified in wholly agricultural environments, as well as in air, water, and soil, have a long life in the environment and are very poisonous for honey bees. Furthermore, contamination by diverse pathogens can weaken bees [36]. Pesticide pollution of honey bee goods is projected once honey bee collections die from pesticide introduction. Honey bee collection mortality is regularly convoyed by parts per million (ppm) pesticide residues in beeswax, honey, bee pollen, and late honey bee specimens [35].

2.6. Pesticide application techniques to crops

Bees are commonly dynamic between daybreak and two hours before sundown, and maximum honey bees explore inside of a 1-3 km circle of the beehive, even though they can travel 5km or more in exploration of nectar and pollen once their resident sources are not sufficient [25]. Bees visit plants during their flowering period (bloom), hence, pesticide application during this period has damaging effects on honey bees. So, risks to bees associated with pesticides may be decreased by applying them to the crops at sundown, and during this time honey bees are not seeking their food outside of the beehive [37].

Pesticide application techniques such as directed spray, foliage, Soil, and Broadcast are commonly used in Rwanda. Pesticide application is still a challenge due to farmers' lack of information on pesticide use (dose, rotation, timing, Re-entry, and Pre-harvest) the absence of practical material adapted to farmer level of knowledge, and this in joining to the Crop Intensification Strategy which looks unclear in identifying the importance of sustainable long route crop production. The use of pesticides is not simply the operation of sprayer or duster. It has to be tied to a detailed knowledge of the pest's problematic behavior as well as its environmental behavior [38].

The above several risks present us with approximate signs of the kind of contact that is most hazardous to the various classes of honey bees in the beehive. The primary factor in occurrences, including the demise of foraging worker bees, is spray spread, while assimilation of polluted nectar, pollen and water are the sources of the colony collapse disorder disease which disturbs various beehives around the biosphere, primarily harming the queen and nurse workers. Additionally, the acaricides used to treat Varroa represent a serious threat to bee larvae and, as a result, to the colonies' ability to survive indefinitely. Apiculturists and farmers can create supervision strategies to prevent pesticide affects by being aware of these pressures [25].

2.7. Maximum Residue Limits (MRLs) of pesticides in honey

Since honey bees might be exposed to such pollutants by collecting pollen, nectar, and water, honey bee products may contain residues of plant protection products (PPPs). Through residue monitoring, pesticide residues for plant protection are occasionally discovered in honey bee products, and quantities might vary from one substance to another.

Honey has been deemed to be a meal with animal products in accordance with Code 1040000 is listed under ‒Products of animal origin (terrestrial Animal)‖ in Annex I of Regulation 396/2005. Cattle may typically consume pesticides in one of three ways:

- Following the substance's direct application to the animal,
- Due to the treatment of their environment, and
- Via residues in food sources [39].

Pesticide deposits rising from the usage of veterinary therapeutic products or after lodging (beehive) treatment (cases one and two) have to be considered when setting MRLs for plant health defense products.

In East Africa, there is no specific requirements for pesticide residues in honey or in horticulture products simply they use the standard requirement set by other regulatory bodies like The Codex Alimentarius Commission and the European Commission. According to RS EAS 36:2020: Honey-Specifications, paragraph 6.2. ‒the product shall comply with pesticides and veterinary drugs Maximum Residues Limits for honey established by the Codex Alimentarius Commission.

Table 0-2: Maximum Residue Limits (MRLs) of pesticides in honey

Product	Pesticide residue	Chemical class	MRLs(mg/Kg)
Honey and other apiculture products With a code number 1040000	Abamectin(insecticide)	Macrocyclic lactone	0.05
	Alpha- Cypermethrin	Pyrethroid	0.05
	Profenofos	Organophosphate	0.01
	Metalaxyl (fungicide)	Anilide fungicide	0.05
	Deltamethrin	Pyrethroid (Neurotoxin)	0.03
	Chlorothalonil	Organochlorine	0.05

Source: [40]

Table 0-3: Pesticides and targeted pests

Pesticide	Crop	Pest
Abamectin	Orange, pepper, tomato, Watermelon, cucumber, Soybean	Amy worm, silkworm, green worm
Metalaxyl	trees, ornamental plants	Water-mold Fungi, phytophthora heart rot androot rot in pineapples
Deltamethrin	Coffee, maize, cereals	Whitefly, leaf beetles, mites, true bugs
Chlorothalonil	Fruits, trees, vegetables,	Fungi on fruits, trees,
	peanuts	vegetables and also controls ticks, mildew and fruits rot
Profenofos	Soybean, maize, potato	Whitefly, armyworm, mites
Alpha-cypermethrin	Soybeans, corn, vegetables, wheat...	Cotton, vegeTable pest

Source: [41]

2.8. Analytical techniques for pesticide residues

In the study of residues of pesticides in foodstuffs, numerous approaches have been used. For the case of Abamectin, Alpha-Cypermethrin, Metalaxyl, Profenofos, Chlorothalonil, and Deltamethrin pesticide residue analysis, approaches can be GC-MS-MS (Gas chromatography coupled with tandem mass spectrometric detection), HPLC with matrix solid phase dispersion (MSPD) and diode array detector (DAD), and liquid chromatography mass spectrometer (LC-MS) [42]. Also, the pesticide residues here can be analyzed by HPLC with an ultraviolet detector because of their consistency, capacity to evaluate Abamectin, Alpha-Cypermethrin, Metalaxyl, Profenofos, Chlorothalonil, and Deltamethrin with a broad range of column materials, and also because of their availability [43].

2.9. High performance liquid chromatography (HPLC)

Due to the variable solute retention by the stationary phase, high-performance liquid chromatography is a method for isolating, identifying, and quantifying the components of a solution in a mobile phase. This technique of analysis is particularly appropriate for compounds which are not simply volatilized, are thermally unstable and have high molecular masses [44]. Ultraviolet (UV) detectors are the most regularly used detection technology because they have a large linear range, are sensitive, and are only mildly impacted by temperature changes. In contrast to GC, HPLC does not require a high vapor pressure in the sample. High-Performance Liquid Chromatography is therefore suitable for both the separation of components with large molecular weights and those with lower molecular weights. Mild conditions, typically ambient temperature, and common solvents like water, hexane, and acetonitrile, are ideal for HPLC [42]. HPLC is an analytical system that separates compounds by using modifications in the delivery of compounds among two non-miscible phases, named the mobile phase and stationary phase. The liquid traveling over the particles is referred to as the mobile phase, and a thin layer that forms on the surface of tiny particles is referred to as the stationary phase. Depending on its solubility in the phases and/or molecular size, each component in a sample has a specific distribution equilibrium under a specific dynamic circumstance.

As a result, the components travel across the stationary phase at various speeds and become disassociated from one another. A depiction diagram of an HPLC device is given away in Figure 3 below.

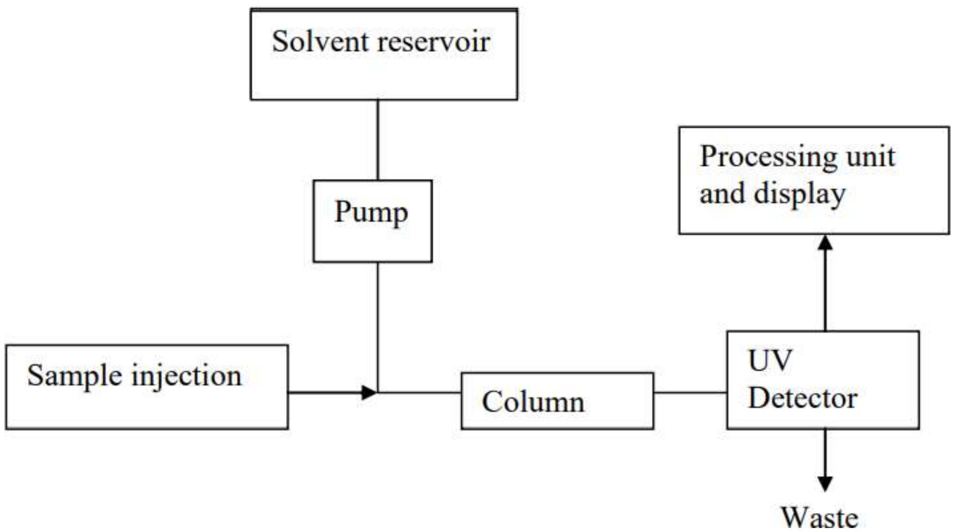

Figure 3:HPLC apparatus with UV detector represented in a diagram

Source: [2]

CHAPTER 3. MATERIALS AND METHODS

3.1. Samples collection

Savanna, grasslands, and wetlands make up the Eastern Province region. The southeast border is formed by lowlands and lakes in the Akagera River basin, and the Eastern border is formed by the Akagera National Park [45]. The sampling was conducted at Kabare, Ndego and Kabarondo Sectors in Kayonza District.

Table 0-4: Beekeepers in Kabare, Ndego and Kabarondo Sectors in Kayonza District

No	Beekeeper	Number of bee hives	Number of apiaries
1	DUTUBURE UBUKI	108	4
2	COPROMA	423	9
3	ABATAVOGERWA	49	3
4	IMPALA	85	3
5	COPANYAKA	183	6
6	Innocent NZABONIMPA	90	5
Total		**938**	**30**

Using the Taro Yamane method, the sample size was determined at a 95% confidence interval.

$$n = \frac{N}{1 + Ne^2}$$

Where

n: is the sample size,

N: indicates the total population and

e: indicates the error margin [46]

And sample size is then calculated,

$$n = \frac{30}{1 + 30(0.05)^2} = 27.90 \approx 28$$

A composite sample is designed by actually mixing portions of separate samples of honey (One sample from one apiary) and this has the benefit of:

- Reducing the total number of samples that need to be analyzed, saving time and costs;
- Providing a representative sample of conditions over a larger area.

In the assessment of the pesticide residues in honey from Kayonza, 28 samples of multifloral fresh honey from *Apis Millifera L.* were collected from three sectors of Kayonza District. 10 samples of honey were collected from Kabare, 10 samples from Ndego and 8 samples from Kabarondo. These samples were collected from different Cooperatives (COPANYAKA, DUTUBURE UBUKI, COPROMA, IMPALA, ABATAVOGERWA, and an individual called Innocent NZABONIMPA). These samples were collected, brought to the lab, and kept there between 4 and 10^0C until analysis. For the present study, the map of Eastern Province and sampling sites were prepared according to the systematic collection of samples.

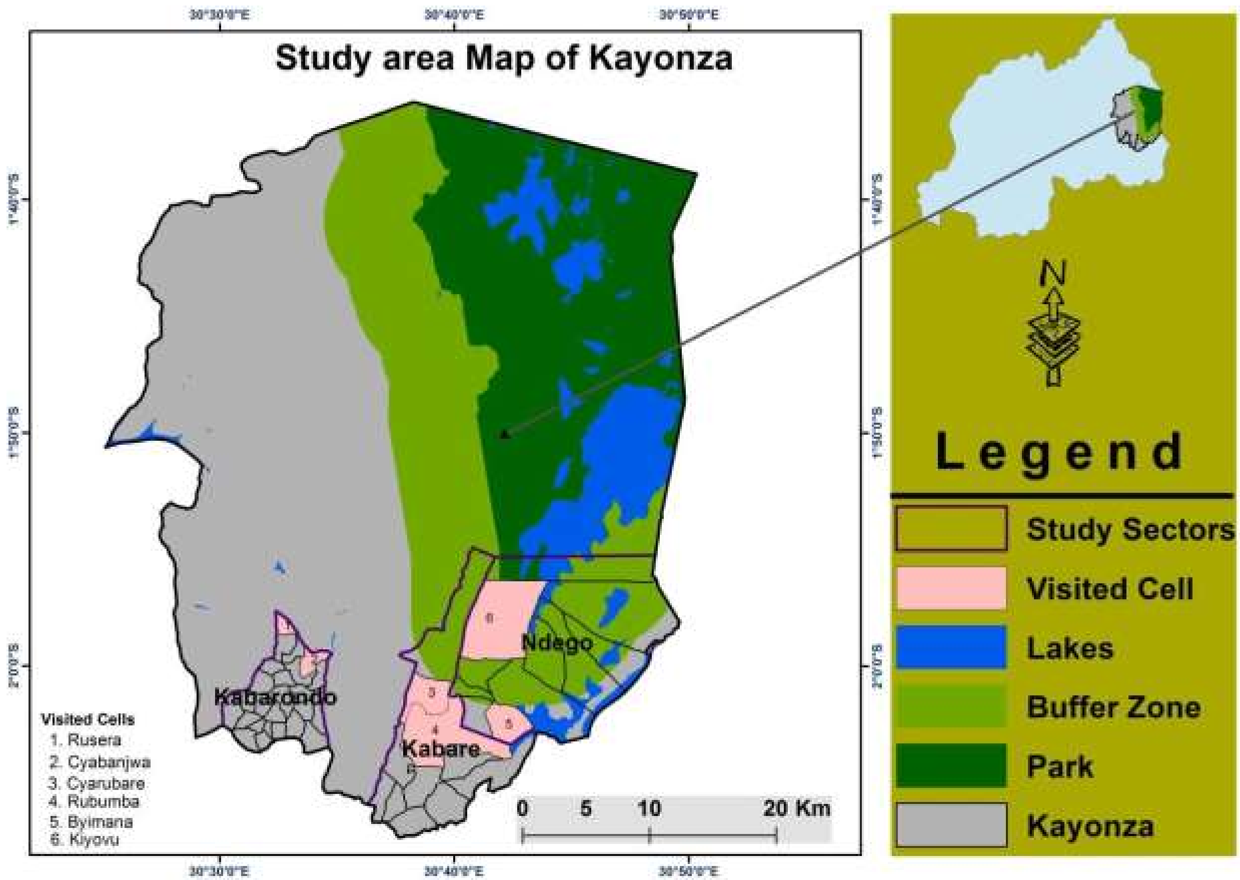

Figure 4: Study area at Kayonza District

3.2. Pesticide residues determination in honey

3.2.1. Reagents, solvents and standards

Pesticide analytical standards that were used and their purity is given in the Table 5 below.

Table 0-5:Purity of standard pesticides

Pesticide	**Percentage purity (%)**
Abamectin	95.5
Alpha-Cypermethrin	99.0
Chlorothalonil	99.7
Metalaxyl	99.1
Profenofos	99.4
Deltamethrin	98.6

Acetonitrile HPLC grade, distilled water-HPLC grade, ethyl acetate, anhydrous sodium sulphate, C18-bonded column analytical silica gel (50 µm) were also used.

3.2.2. Residues Extraction

For the extraction of pesticide residues, Muhammad Aslam Farooqi's method [34] was employed with a few modifications. 5g of honey were weighed in a flask. The honey sample was combined with 5 ml of distilled water and homogenized by shaking to lower the viscosity and make it easier to handle. Next, the sample was combined with 50 ml of ethyl acetate (solvent) and extraction was carried out by stirring on a rotating lab shaker for 20 min. After extraction, the separation was followed by centrifugation at 2500 rpm for 10 minutes, and the organic phase was collected at the upper part. The supernatant was run up, and the residues were again subjected to extraction using 40 ml of the same ethyl acetate solvent. In a rotary evaporator operating at 45°C under reduced pressure, the solvent was evaporated. The leftovers were then diluted in 5 ml of acetonitrile and filtered through a Polytetrafluoroethane (PTFE) filter with a 0.50 µm pore size to produce filtrate that needed to be cleaned.

3.2.3. Clean-up of samples

The honey samples subjected to extraction (about 5ml) were then prepared by using a mixture of 5g of silica gel and 1g of anhydrous sodium sulphate. The extracted samples were filtered by being run through a chromatographic column.

Then, elute was then secured. Using HPLC this elute was examined [47] [48].

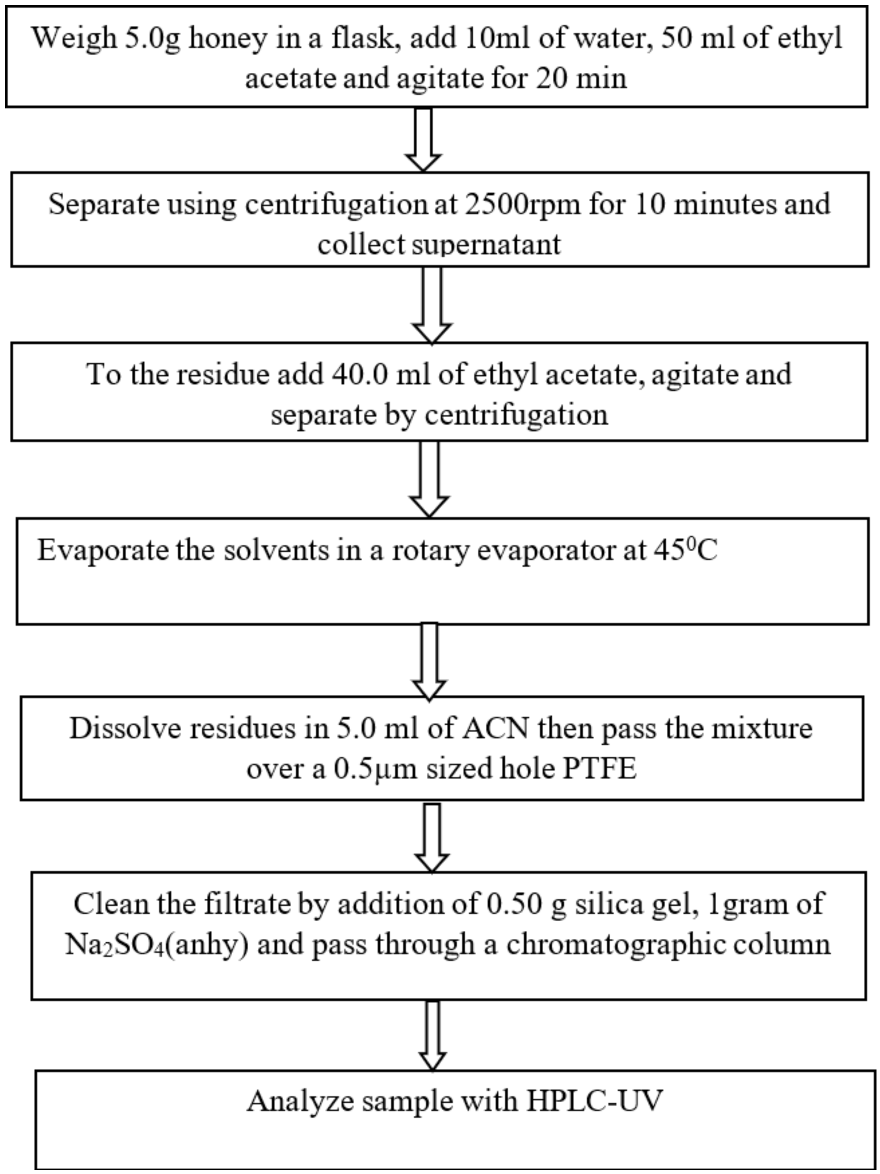

Figure 5:Scheme of the extraction process

3.2.4. Mobile phase preparation

For HPLC, a mobile phase liquid is constantly added, which elutes sample constituents successively based on their attraction to the stationary phase. The separate constituents can be identified through a detection system [44]. HPLC-grade water and acetonitrile were mixed in 60:40 volume/volume proportions.

3.2.5. HPLC-UV instrumentation and operating conditions

Pesticide residues in honey were evaluated using a High-performance Liquid Chromatography-UV detector system [49] [34]. The system of Shimadzu HPLC-UV consisted of a degassing unit (DGU20A$_5$), liquid chromatography (LC-20AD pump), communication bus module (CBM-20A), and UV/Vis detector (SPD-20A), which interacted with the program LC solution. It included a 250 mm-long reversed phase C-18 analytical column, 4.6mm inner diameter and small particles of 5.0µm size. A 30^{0}C temperature was maintained. The injection of the sample volume was 20 µL. A mobile phase is the mixture of acetonitrile and ultra-pure water (HPLC grade) in 60: 40 proportion. A 1.2 mL/min flow rate was maintained. The investigation of each pesticide by HPLC-UV detector has shown that the maximum absorbance was 230nm [49]. These instrumental conditions are chromatographic separation parameters.

3.3. Method validation

A method validation is the procedure of defining an analytical requirement, and approving that the method under consideration has performance abilities reliable with what the application needs [50]. Analysis reliability is ensured via method validation. The variables accuracy, precision, linearity, and limits of detection (LOD) and quantification (LOQ) were all taken into account in this investigation. Recovery tests and sample spiked at two different levels of 0.01 and 5 ppm with known concentrations of the pure standard solution were used to assess the accuracy of the method. Extraction and cleanup were completed as previously mentioned. Calculations were made to determine the amount of each pesticide in the final extract. In order to evaluate the precision of extraction and cleanup, recovery studies were carried out. The fact that the European Commission required these pesticide recovery tests shows that the procedure can be reliable and precise when relative standard deviations (RSDs) don’t exceed 20% and the precision of the data acquired is between 70 and 110% with [47]. Prior to the analysis of samples, this was carried out daily. Different known concentrations (0.001, 0.01, 1, 10 and 50µg/mL) that were made by dilution of the stock solution, were used to test the linearity. To confirm that there were no pesticide residues in the validation, the blank samples were also examined.

3.4. Identification and quantification

By contrasting the sample peaks' retention times with the standard peaks as well as the amount of residue noted in the integrator chart, the chemical compounds that make the pesticide residues were evaluated. The equation used to calculate the residual level of pesticides in $^{\mu g}/_{g}$ (ppm) is as follows:

$$Pesticide\ residues\ \text{in}\ ppm = \frac{Asa. Cs. V}{As. \text{W}}$$

Where,

Asa: sample peak area**;**

As: standard peak area;

Cs: standard concentration, $^{\mu g}/_{ml}$

W: Sample weight in grams

V: Sample volume overall in ml

The sample concentration assessed by this study was compared to a known spiked sample concentration in the recovery case.

C found= concentration of sample allocated by analysis, µg/g

C spike= spiked sample concentration, µg/g

$$Recovery(\%) = \frac{Conc.found}{Conc\ spiked} * 100$$ **[47].**

3.5. Statistical Analysis

Microsoft Excel 2019 was used to do statistical analysis on the found results [51].

CHAPTER 4. RESULTS AND DISCUSSIONS

The assessment of pesticide residues in honey from Eastern Province was studied under laboratory conditions. The findings showed that only a little percentage of samples of honey had pesticide residues in them.

4.1. Linearity and range

Calibration curves using pesticide standards were created to determine where a pesticide maintains a linear relationship between the analyte concentration and the HPLC-UV response. Considering the Food and Drug Administration Office of Regulator's validation process [52], the linearity of the method is reflected satisfying when R^2 is between 0.96-1.0. All of the pesticides were fulfilled (Annex 3). According to the Food and Drug Administration's validation principle, the R^2 values (Table 5) are helpful for the quantification. These values maintain the outcomes of the recovery tests as they approve the accuracy of the technique. The dilute concentrations were prepared: 0.001, 0.01, 1.0, 10, and 50 ppm for each standard. The HPLC was used to analyze each of these solutions, and peak regions were plotted against pesticide concentration (annex 3).

4.2. Limits of Detection (LOD) and quantification (LOQ) (mg/L)

The limit of detection (LOD) was established as the lowest concentration giving a response that is three times the baseline noise based on the examination of the control (untreated) sample. The limit of quantification (LOQ) was defined as the lowest concentration of a given pesticide that results in a reaction that is 10 times the background noise [47].

LOD and LOQ are expressed as ppm (mg/L) of solution; R^2, linearity greater than 0.97 and P-value not as much of than 0.05 shows statistical significance of the results between variable and response, therefore, according to [53], R^2 and P here are in a good range. Five samples of honey that had been spiked with target pesticides were examined to determine the LOD, which was then calculated using the formula.

$$LOD = 3.3 * \frac{SD}{S}$$

Where:

SD standards for standard deviation of calibration curve's peak area

S represents the calibration curve's slope and 3.3 is the one-tailed t value at a 99% confidence level. In order to determine the LOQ using the formula, the calculation procedure is once again based on the standard deviation of the response (S.D) and the slope of the slope of the calibration curve(S):

$$LOO = 10 * \frac{SD}{S}$$

Where **SD** standards for standard deviation of calibration curve's peak area

S represents the calibration curve's slope. The Standard Deviation (S.D) values of and slope were obtained from the functions (Table 6), when creating calibration curves in the Excel.

Table 0-6: LOD and LOQ in mg/L for analyzed pesticides

Pesticide	**LOD**	**LOQ**	**Equation**	**Linearity (R^2)**	**P-value**
α-cypermethrin	0.049	0.147	y = 12310x + 192231	0.9748	0.03
Abamectin	0.0018	0.0061	y = 591932x - 134696	0.9988	0.01
Chlorothalonil	0.012	0.041	y = 110317x + 126820	0.9937	0.05
Metalaxyl	0.014	O.046	y = 49371x + 35383	0.998	0.009
Deltamethrin	0.002	0.007	y = 139261x - 9956.5	0.9987	0.008
Profenofos	0.028	0.085	y = 367286x + 440209	0.9835	0.04

4.3. Accuracy (Percentage recoveries)

The closeness between the true value and the found value is how accurately an analytical procedure performs. The accuracy of an analytical procedure is determined by its level of precision. The ratio of the amount of analyte detected to the amount of analyte recovered after spiking samples in a blank is the accuracy. The RSD of the replicates provides an indication of the test method's correctness and delivers the analysis deviation. The average of the replicates stated as a percent (%), indicates the accuracy of the test method. Samples of honey were spiked with two distinct concentrations of 0.001mg and 0.5mg/L of standard pesticide. Results have demonstrated that the current approach for spiked samples recovered well (going from 90.9 to 72.3%) with regard to the six pesticides that have a relative standard deviation under 20%. These results were acceptable and met the European Commission's SANCO/12571/2013/Quality Control and Validation-EU requirements that a method's accuracy percentage of the data be between 70 and 110% and the RSDs be less than 20%.

$$RSD = \frac{SD}{Mean} * 100$$

Where

SD standards for the standard deviation and Mean is the mean of peak area [54].

The HPLC system was standardized to check its performance. The analysis of the pesticide standard solutions was performed, and the findings below showed that the tool and established circumstances were suitable for examining pesticide residues in samples of honey.

Table 0-7: Pesticide Residues recoveries and RSDs

Pesticide	**Recovery (accuracy percentage)**	**RSDs (%)**
Alpha cypermethrin	90.9	16.3
Abamectin	72.3	7.7
Chlorothalonil	89.7	7.0
Metalaxyl	74.8	5.65
Deltamethrin	73.7	9
Profenofos	89.0	9.13

The values of recoveries and relative standard deviation (RSD) are expressed in terms of percentage. The approach is deemed suitable for residue determination when the RSD is less than 20%. Recoveries and RSDs obtained are in a good range with recoveries greater than 70% and RSDs less than 20%, based on the requirements of EU-Commission SANCO/12571/2013/Quality Control and Validation.

4.4. Pesticide Residues in Honey samples from NDEGO Sector

Findings of pesticide residues identified in honey samples from diverse areas of the NDEGO Sector of Kayonza District indicated that three samples were polluted with residues of Abamectin 0.048, 0.034, and 0.024 mg/kg and one only sample of honey was contaminated by Deltamethrin 0.014 mg/kg. These results do not exceed MRLs, but the pesticide residues of Profenofos, Alpha-cypermethrin, Chlorothanil and Metalaxyl were Below Limit of Detection (*) in all samples from this region of Ndego Sector (Table8).

Table 0-8: Pesticide Residues in Honey from NDEGO Sector

Pesticide & Ndego MRL	Abamectin	α-Cypermethrin	Chlorothalonil	Metalaxyl	Deltamethrin	Profenofos
	0.05 ppm	0.05 ppm	0.05 ppm	0.05 ppm	0.03 ppm	0.01 ppm
NDG1	*	*	*	*	*	*
NDG 2	*	*	*	*	0.014 ± 0.003	*
NDG 3	0.048 ± 0.002	*	*	*	*	*
NDG 4	*	*	*	*	*	*
NDG 5	0.034 ± 0.003	*	*	*	*	*
NDG 6	*	*	*	*	*	*
NDG 7	*	*	*	*	*	*
NDG 8	0.024 ± 0.001	*	*	*	*	*
NDG 9	*	*	*	*	*	*
NDG10	*	*	*	*	*	*

Results are stated as means ± standard deviation, and whereas, * stands for below limit of detection and NDG stands for NDEGO. The results obtained show that several samples of honey had pesticide residues. The samples of honey collected from the Ndego sector had the highest levels of contamination with pesticide residues. This is due to the large practice of pesticides in this region dedicated to a maize crop. In Kayonza District-Ndego Sector, maize is the main crop, followed by soyabeans, tomatoes, and chili peppers. And only maize is the crop that has been selected in the buffer zone of Ndego Sector. In the Ndego Sector, there is a BRAMIN plantation. BRAMIN is a joint project between Bralirwa and Minimex, and it is situated along Lake Ihema to produce maize and soybeans. The farm of BRAMIN is about 650 hectares in the Ndego Sector. Maize and soybeans are rotated on a 6-month and 3-month cycle, respectively. For this crop there are a certain number of pests and associated effects that are usually taken into consideration as the most important constraints in production; nevertheless, depending on the habitat and farming practices, they have varying degrees of pestiness. Maize stalk borers (such as Busseola fusca), maize stripe virus, leaf blight, striga weeds, and storage pests are the most significant maize pests and diseases. Currently, diseases like leaf blight and maize streak are under control by means of tough varieties and cultural practices. Pesticides like Abamectin, Deltamethrin, alpha-cypermethrin, Chlorothalonil, and others are applied to maize, soyabeans, tomatoes, farms in Ndego sector, Kayonza District. And also, the location of honey bees is closed to the farm activities of BRAMIN which is the cause of pesticide residues in honey.

4.5. Pesticide Residues in Honey samples from KABARE Sector

Findings of pesticides identified in samples of honey from different sites in the KABARE Sector of Kayonza District indicated that one sample was polluted with residues of Deltamethrin at 0.015ppm. And this sample's result does not exceed the maximum residue limit set by MRL (Table 2), but the pesticide residues of Abamectin, Profenofos, Alpha-cypermethrin, Chlorothanil and Metalaxyl were below the LOD for all samples from this region of Kabare Sector (Table 9).

Table 0-9: Pesticide residues in honey samples from KABARE sector

Pesticide & MRL / Kabare	Abamectin	α-Cypermethrin	Chlorothalonil	Metalaxyl	Deltamethrin	Profenofos
	0.05 ppm	0.05 ppm	0.05 ppm	0.05 ppm	0.03 ppm	0.01 ppm
KBR1	*	*	*	*	*	*
KBR 2	*	*	*	*	*	*
KBR 3	*	*	*	*	*	*
KBR 4	*	*	*	*	*	*
KBR 5	*	*	*	*	*	*
KBR 6	*	*	*	*	0.015 ± 0.001	*
KBR 7	*	*	*	*	*	*

Results are stated as means ± standard deviation, and whereas, * stands for below limit of detection and KBR stands for KABARE. The results obtained show that one sample of honey had residues of pesticides. The pesticide residues at a high level were detected from the honey samples that were collected from the Kabare Sector due to the minimum practice of pesticides in this region. In this region, the suitable crops are banana, cassava, coffee, and low-lying maize. Generally, there is no intensive agriculture and no use of pesticides. And also, the location of honey bees is far away from the agriculture zone.

4.6. Pesticide Residues in Honey Samples from KABARONDO Sector

Results of pesticide residues in honey from different areas of Kabarondo Sector of Kayonza District indicated that no sample was polluted with residues of the following pesticides: Deltamethrin, Abamectin, Profenofos, Alpha-cypermethrin, Chlorothalonil, and Metalaxyl (Table 10).

Table 0-10: Pesticide residues in honey samples from of KABARONDO

Pesticide & MRL / Kabarondo	Abamectin	α-Cypermethrin	Chlorothalonil	Metalaxyl	Deltamethrin	Profenofos
	0.05 ppm	0.05 ppm	0.05 ppm	0.05 ppm	0.03 ppm	0.01 ppm
KRD 1	*	*	*	*	*	*
KRD 2	*	*	*	*	*	*
KRD 3	*	*	*	*	*	*
KRD 4	*	*	*	*	*	*
KRD 5	*	*	*	*	*	*
KRD 6	*	*	*	*	*	*
KRD 7	*	*	*	*	*	*
KRD 8	*	*	*	*	*	*

KRD stands for KABARONDO and * stands for below limit of detection. In this sector and also in ther sectors in the same region, like Nyamirama, Mukarange in the District of Kayonza, banana plantations are mostly cultivated. The results obtained show that no sample of honey contained pesticide residues. The pesticide residues were not sensed in honey samples that were gathered from the Kabarondo Sector due to the low practice of pesticides in their non-developed farming in this region. The pesticides commonly applied are Mancozeb, Metalaxyl, Chlorothalonil, Alpha cypermethrin for the crops tomatoes, sweet potatoes, and soja beans that are present in this sector. Honey bees are distributed in small forests within the farming areas.

4.7. Discussion on pesticide residue in honey from Eastern Province, Kayonza District

Numerous categories of pesticide contamination in many commodities used as food, as well as honey, are important hazards for the population. Controlling the pesticide content in honey is essential for enhancing health since the number of people using pesticides in farming is increasing in the recent past [55]. *A. mellifera*, the raw honey samples were gathered from various sectors of Kayonza District, Eastern Province of Rwanda for determination of pesticide residues by HPLC-UV.

The results achieved have shown that very few honey samples were polluted with pesticide residues, but less than the MRLs. The sampling areas that showed contaminated samples were observed in the Ndego sector due to extensive agriculture of maize, Chili peppers, soybeans, and tomatoes in the region that requires application of pesticides to combat worms, Sucking pests, beetles, aphids, and spider mites [56].

The honey samples from Ndego sector contain pesticide residues, which include Deltamethrin and Abamectin. Out of 6 pesticides studied, just residues of two pesticides (abamectin and deltamethrin) were identified in a range between 0.014 and 0.048 mg/Kg and were below the maximum residue limits of abamectin and deltamethrin, 0.05 and 0.03mg/Kg, respectively (Table 2) according to EU food safety [57] and have no effect on any biological things or humans.

A number of scientists have studied pesticide pollution in honey samples gathered from various areas of the biosphere to detect pesticide residues. The findings of different researchers from the biosphere agree with the outcomes of current research since there have been numerous reports of pesticide residues in honey products. However, the contents can be different due to the use of different techniques for analysis and sample collection from different sources [17].

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

It has been assessed that above 80% of all plants bearing flowers and fruits are subject to honey bees, as cross-pollinators and pesticide applications on different flowering plants have an impact on different species of pollinators as well as honey bee foragers once they interact with these flowering plants treated with pesticides for pest protection [47].

5.1. Conclusions

Plant protection in this moment by the use of pesticides is a key point, as they are essential for a farm's yield. Only a solution to the issues related to pesticide residues is to find method to reduce the hazard of pesticide residues honey bees because this affect globally the agricultural production.

In the assessment of residues of pesticides from raw honey, it is been determined that residues of abamectin were detected in three samples of honey from the Ndego Sector and residues of Deltamethrin in one (1) and one (1) honey sample from the Kabare sector. Honey samples that had pesticide residues represented 17.8% of the considered honey samples, but their levels were not greater than the Maximum Residue Limits, which are tolerable residue levels according to European Commission (EC) Regulation No 396/2013 [58]. The results from this research make known that the intensities of the pesticide residues identified from the region under research were in the range from Below the detection limit to 0.048 mg/kg for abamectin and from Below the detection limit to 0.015 mg/kg for Deltamethrin. Honey with these results can be accepted at EU market under the fulfillment requirement on pesticide residues. Therefore, it can be concluded that the honey samples used in this research are safe for consumers. The residues of profenofos, Chlorothalonil, Metalaxyl, and Alpha-cypermethrin were not perceived in any honey sample from the Kayonza District.

5.2. Recommendations

Even if pesticides have a positive impact on agriculture and health parts to regulate diseases and pests in tropical states like Rwanda, they have been confirmed to disturb non-target creatures as well as humans. The residue monitoring scheme is a requirement so that Rwanda may join the Third countries from which the EU may import honey. For that reason, to protect honey consumers and to ensure good practice of pesticides in the regions where apiculture is located, some of the following recommendations need to be taken into consideration:

To Government

1) There is a need for periodic quality control mechanisms of pesticide residues in food products using laboratory equipment like GC-MS and HPLC-MS.
2) The regulatory bodies in charge of inspection, RICA and Rwanda FDA, should organize training sessions to upgrade the knowledge of farmers on how to implement Integrated Pest Management (IPM) technology; this can considerably decrease pesticide use without decreasing production. The pesticide business needs to be more environmentally friendly, with more highlighting on Agro-ecological products. These involve an approach that enables the exchange and collaboration among stakeholders (farmers, beekeepers, authorities, and producers, and sellers)
3) Guidelines for primary metabolites of pesticides are necessary because they must be handled separately since the metabolites of pesticides can be more toxic than the initial preparation of the pesticide. Pesticides have to be applied in farms according to recommended measures with good agricultural practices (GAP). Monitoring campaigns should be extended to many sources: farms, markets, raw honey, processed honey, etc.

To local community

4) Reducing the use of pesticides for the duration of blooming phases of plant life and not spraying when wind is blowing on the way to regions where bee hives are sited.
5) Farmers should be mindful of the harms that pesticides demonstrate for honey-bees and honey products including honey. Because a large variety of pollinator species, in addition to honey bees, may also be impacted, farmers should prevent chemical pollution of sites, particularly water bodies.

6) In cooperation, the farmers and beekeepers should be educated appropriately about pesticide application methods and how to decrease pollution in a specific region. At sundown or early sunrise, application of pesticides is continuously needed since foraging bees are at that time in the hive and out of risk. Also, preserve bee colonies away from the treated grounds as far as possible.

Areas for further studies

7) Based on the search results, here are some potential areas for further studies on pesticide residues in honey from Kayonza District, Rwanda

- ❖ Investigate a wider range of pesticides beyond the 6 studied (abamectin, profenofos, alpha-cypermethrin, chlorothalonil, deltamethrin, metalaxyl) to get a more comprehensive understanding of the pesticide contamination profile.
- ❖ Analyze for potential metabolites and breakdown products of the pesticides, as they may also pose risks
- ❖ Conduct longitudinal studies to monitor pesticide residue levels in honey over multiple seasons or years to identify any trends or seasonal patterns.
- ❖ Evaluate the impact on bee health and productivity:
- ❖ Investigate the potential sublethal effects of the detected pesticide residues on the health, behavior, and productivity of honeybees in the region.

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ANNEXES

Annex 1: Pesticides applied in Rwanda in 15 years in tones (source FAOSTAT,2021)

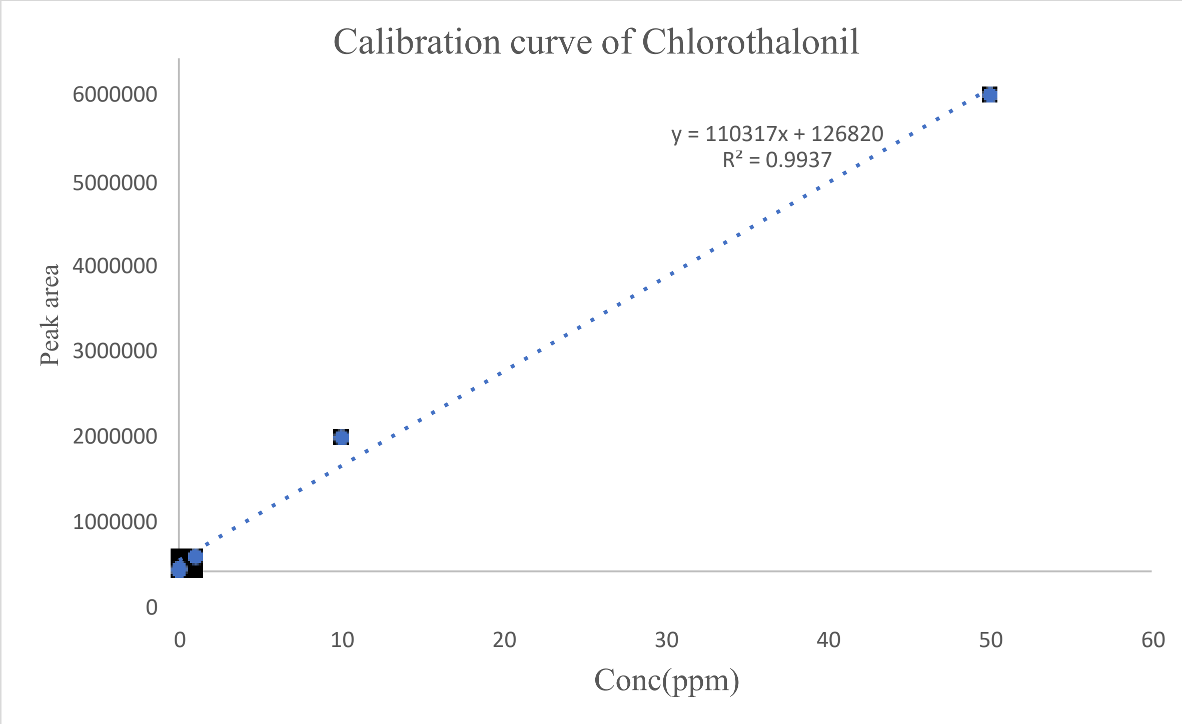
YEAR	Total	Insecticides	Herbicides	Fungicides	Organophosphates	Pyrethroids	Dithiocarbar Mates
2006	289	64	0	225	49	1	217
2007	398	75	7	316	41	32	310
2008	322	51	0	271	39	10	270
2009	1188	95	1	1091	74	18	1043
2010	954	63	1	889	50	8	801
2011	182	68	9	105	47	19	100
2012	926	91	10	816	13	60	746
2013	1842	333	27	1480	24	147	1413
2014	2061	234	9	1818	38	138	1717
2015	2484	309	6	2168	68	132	1954
2016	2027	317	1	1709	53	207	1627
2017	2027	317	1	1709			
2018	2027	317	1	1709			

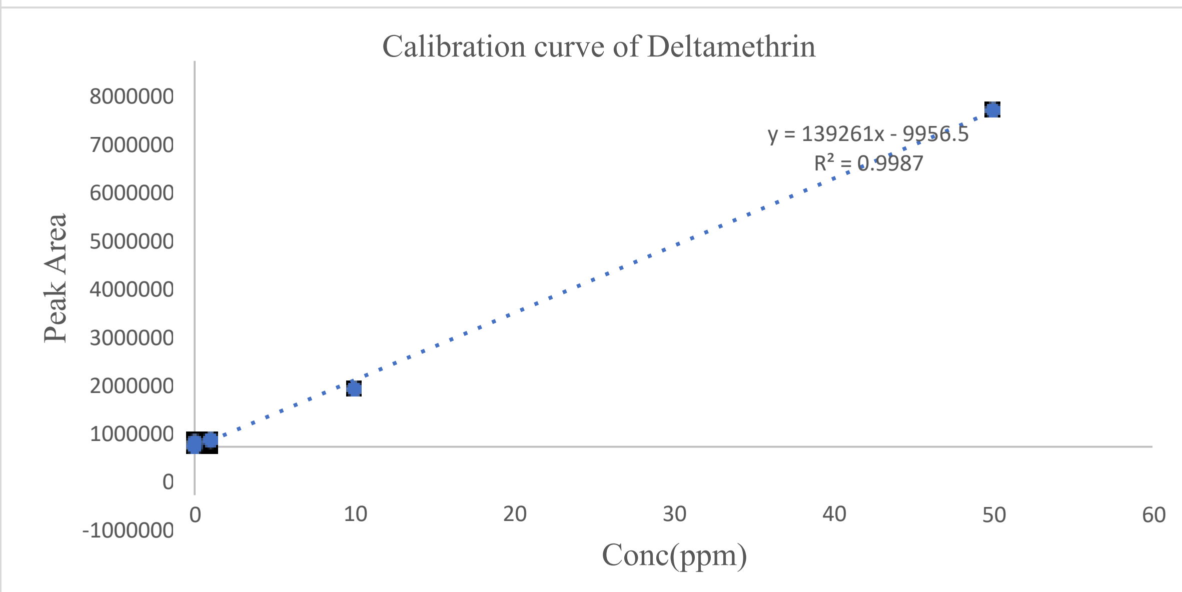
Annex 2: Pesticide standard solutions and their response (peak area) for method validation

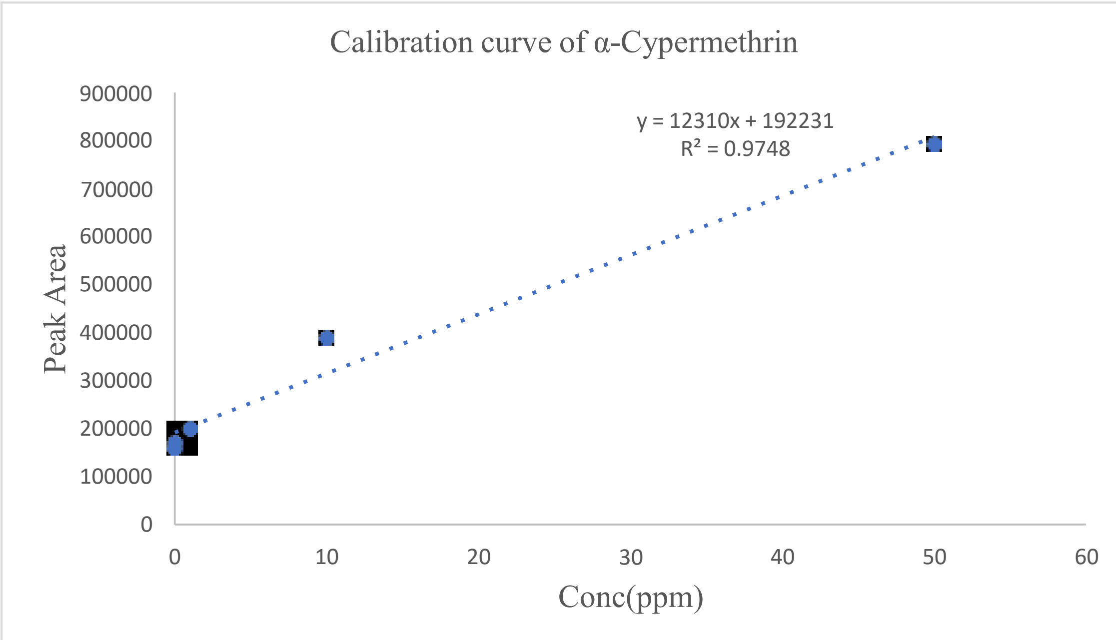
Standards concentration in ppb	METALAXYL	CHLOROTHALONIL	DELTAMETHRIN
	RT:0.746	**RT:1.278**	**RT:12.751**
2	17667	14464	14680
10	27906	35939	98685
1000	105537	169879	142944
10000	535858	1568049	1201461
50000	2502153	5576416	6989021

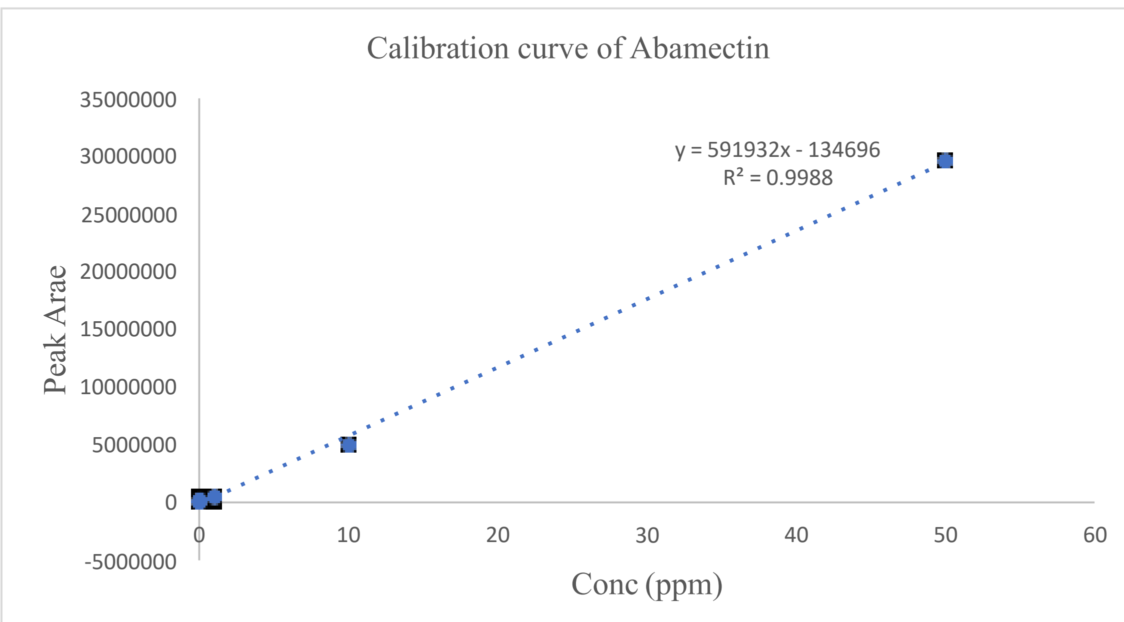
Standards / Concentration in ppb	ALPHA CYPERMETHRIN	ABAMECTIN	PROFENOFOS
	RT: 1.32	**RT:3.47**	**RT:5.72**
2	159467	58926	4674
10	170613	238624	19724
1000	199669	507829	193181
10000	389456	5022953	5941014
50000	793018	29613154	18451321
Blank retention time: 1.006			

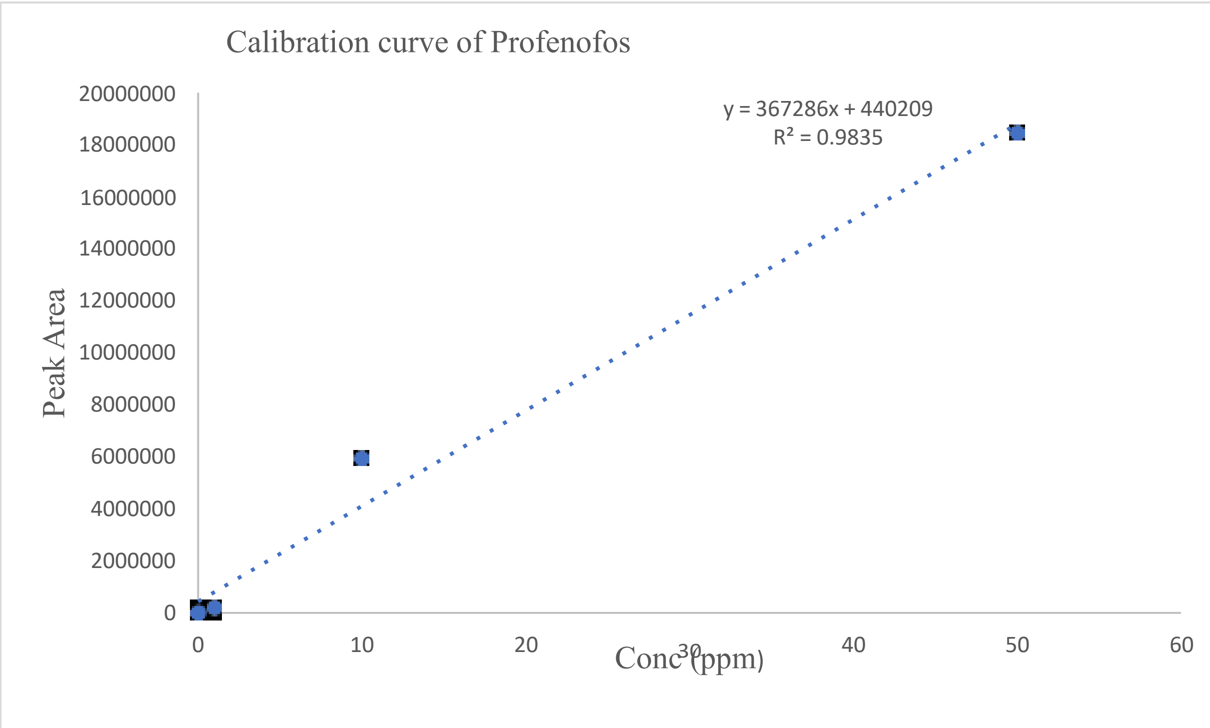
Annex 3: Calibration curves of analyzed pesticides

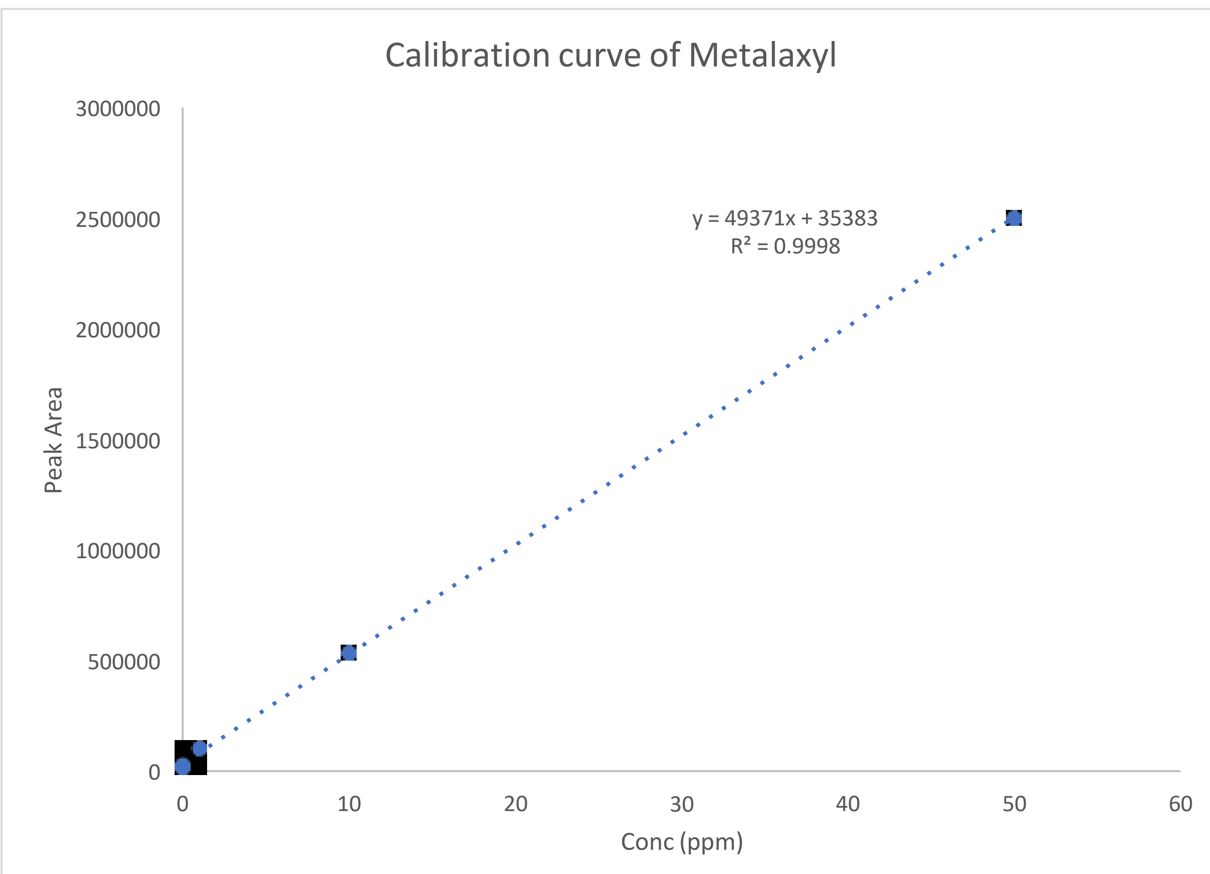




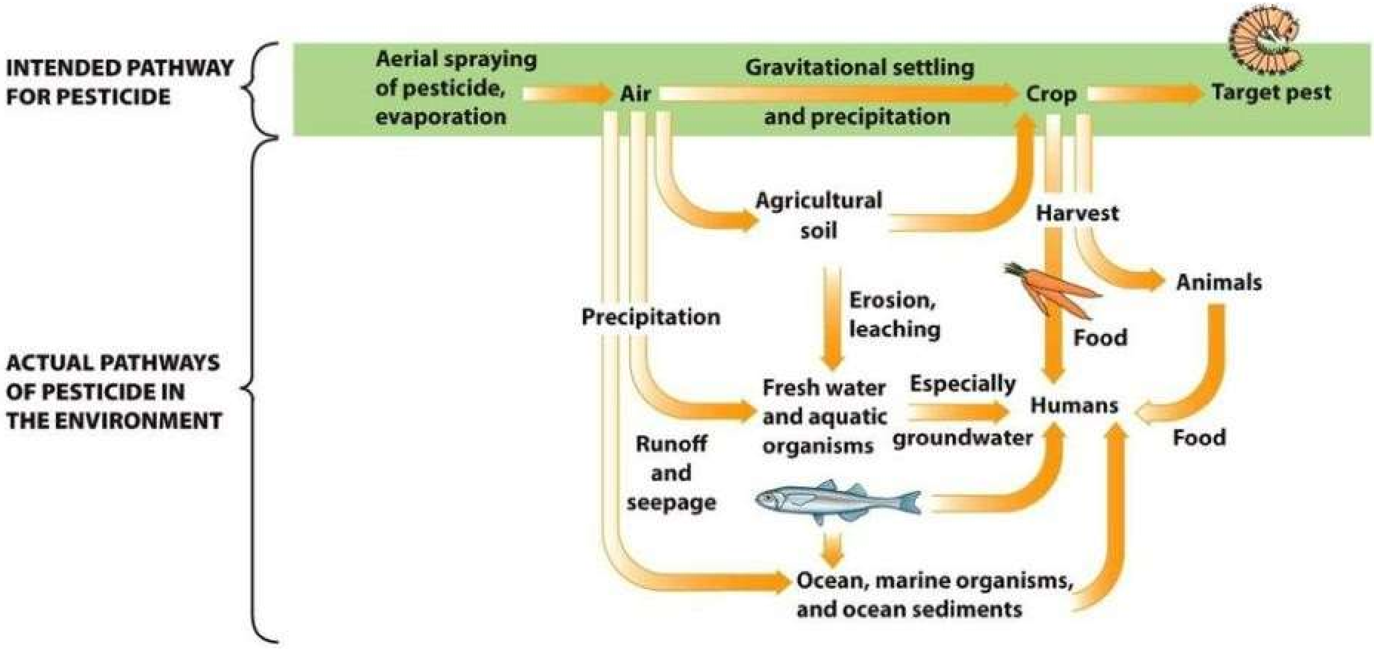

Calibration curve of α-Cypermethrin
y = 12310x + 192231
R² = 0.9748
Peak Area
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
0
10
20
30
40
50
60
Conc(ppm)


Calibration curve of Abamectin
y = 591932x - 134696
R² = 0.9988
Peak Arae
35000000
30000000
25000000
20000000
15000000
10000000
5000000
0
-5000000
0
10
20
30
40
50
60
Conc (ppm)


Calibration curve of Profenofos
y = 367286x + 440209
R² = 0.9835
Peak Area
20000000
18000000
16000000
14000000
12000000
10000000
8000000
6000000
4000000
2000000
0
0
10
20
30
40
50
60
Conc (ppm)

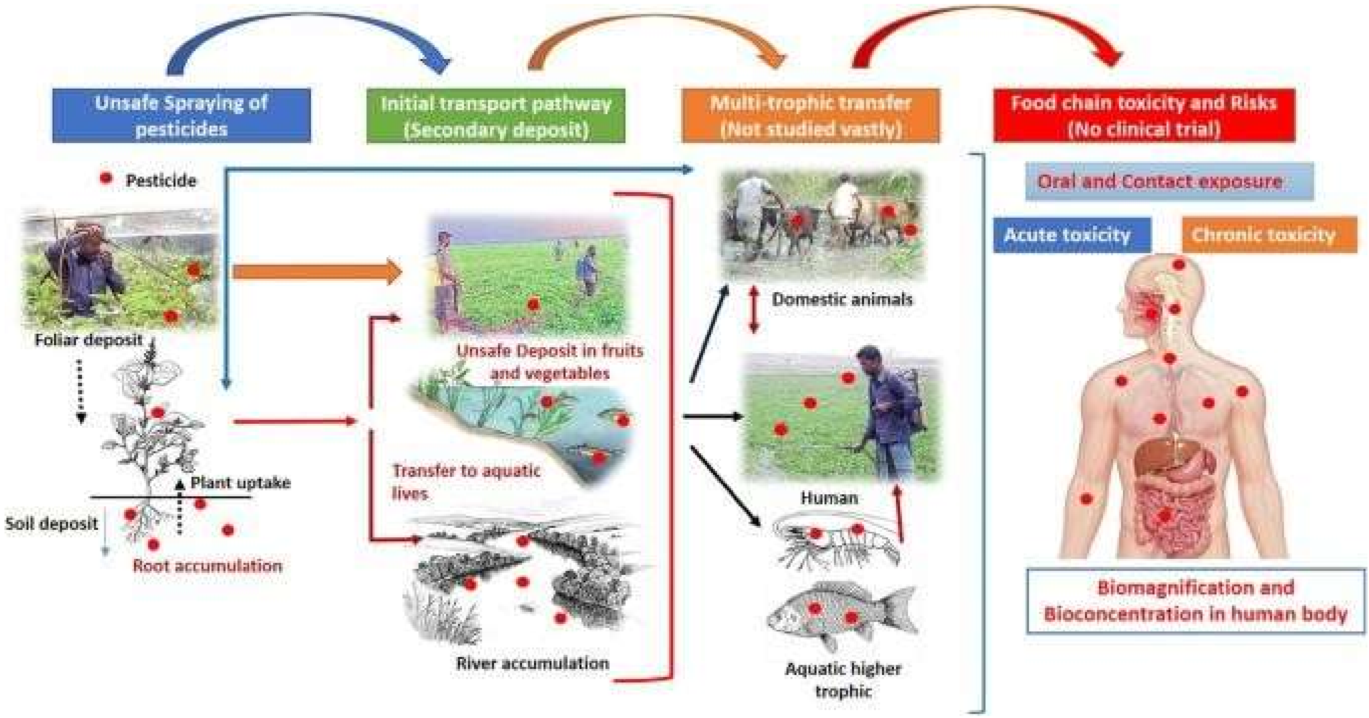

Calibration curve of Metalaxyl
y = 49371x + 35383
R² = 0.9998
Peak Area
3000000
2500000
2000000
1500000
1000000
500000
0
0
10
20
30
40
50
60
Conc (ppm)

Annex 4: Pesticide mobility in environment



Source: [59]

Annex 5: Human Health risks due to pesticides



Source: Quality Assurance of Honey [60]