



GSJ: Volume 11, Issue 6, June 2023, Online: ISSN 2320-9186

www.globalscientificjournal.com

Assessment of Pesticide Residues in Honey from The Kayonza District, Rwanda

Olivier BAMURANGE

Dr. Jean NTAGANDA (Ph.D.)

Dr. Jean Bernard NDAYAMBAJE (Ph.D.)

Email: bamurangeolivier0@gmail.com

Department of Chemistry, School of Science, University of Rwanda

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 4-10⁰C 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. Traces of abamectin and deltamethrin pesticide residues were found, and the detection ranges were limit of detection (LOD) to 0.048mg/kg for abamectin and LOD 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 the findings of current study 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, Pesticides, HPLC, Limit of Detection, Maximum Residues Limits*

1. INTRODUCTION

Honey is one of the naturally delicious substances made by honey bees from flower head nectar. Honey is a complex natural food and unquestionably the only sweetener that is unprocessed (Tarek, 2020). 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(Alghamdi et al., 2020). 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. Though the dependence on pesticides has caused several ecological problems, including pesticide residues in food, which create a potential hazard to human well-being(Houbraken et al., 2017), 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 (Priyanka, 2020)

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 (Rundlöf et al., 2008).

Organisms on the grounds and in nearby ecosystems have been impacted directly and indirectly by the rising use of pesticides in agricultural activities (Rundlöf et al., 2008). However, the use of 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 (Eissa et al., 2014)

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 7km² 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 (Tarek et al., 2014).

The increase in agricultural production has been significantly aided by the use of pesticides in agriculture, but 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 (Houbraken et al., 2017). 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 (Irungu et al., 2016). The main objective is to investigate the levels of pesticide residues in honey from Kayonza District. 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 (Barganska, 2014). In the last centuries, beekeepers have become more aware of the ecological factors that enable them to successfully raise their honey bee colonies from a wide range of flowering. As honey is a product for human feeding with highly valued by health specialists in the nutrition field, the quality control of honey is important because the quality of 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 (Alehagen, 2011).

1.1. 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 1) (NISR, 2012). 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 (Alghamdi et al., 2020). 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 (WHO and FAO, 2014).

Bees are typically exposed to pesticides through the ingestion of residues found in the pollen and nectar of contaminated plants, such as

weeds around farms or crop plants (Irungu et al., 2016). 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 (sunflowers), 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 (Sanchez-Bayo & Goka, 2016).

Besides pollen and nectar, food for honey bees, these last also drink water to preserve their organism's heat or coolness in normal conditions (Lasheras et al., 2021). Pesticide residues, like other particles in the environmental compartments, move from one place to another 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 (Siebers et al., 2003)). 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 (Irungu et al., 2016).

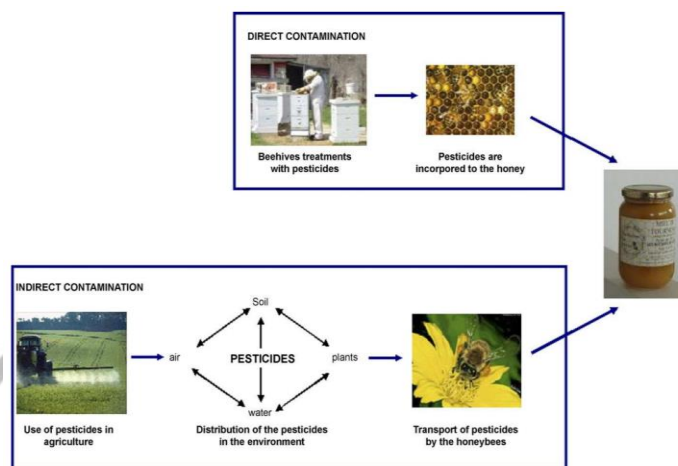


Figure 1: Routes of honey contamination with pesticides

Source:(Nazir et al., 2017)

Honey bees are continually exposed to acaricides (Amitraz, Cymiazole, Flumethrin, Bromopropylate, Coumaphos, and Fluvalinate) in addition to pesticides used in agricultural crops (Sanchez-Bayo & Goka, 2016) to manage parasites such as Varroa. In this instance, honey bees interact with high pesticide residue levels that are present on the waxy cells of comb, primarily impacting the larvae that are still developing (Irungu et al., 2016).

1.2. Pesticide residues in honey and health Hazards

While pesticides are used to destroy pests, some of them can also have negative impacts on human health and the environment. 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 (Aryal et al., 2016). The relations among the wellbeing of environmental service benefactors and anthropological wellbeing continue indefinitely. Through the ancient period, a cumulative various of studies have claimed the positive effects of healthy pollinator societies on anthropological health (Garibaldi et al., 2022). 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 (Wang et al., 2022). By using pesticides in agriculture and apiculture, there is an excessive yield from crops. Actually, pesticides are necessary to meet the global standard requirement for a variety of food goods, and there is no other substitute that can compete with them on such a

large scale. When consumed through various food chains, their slow decomposition and careless use by farmers may cause environmental contamination and harm to humans(Hamilton, 2004).

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 musculo-skeletal pathologies, haematomas, and other diseases are the hazards of pesticide residues in relation to human contact(Farooqi, 2015).

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(Bogdanov, 2006).

1.3. High-performance liquid chromatography (HPLC)

High-performance liquid chromatography is a system for separating, identifying, and quantifying the components of a solution in a mobile phase due to the varied retention of the solutes by the stationary phase. This technique of analysis is particularly appropriate for compounds which are not simply volatilized, are thermally unstable, and have high molecular masses (Sary, 2018). 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 the sample to have a high vapor pressure. HPLC 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(Oyugi, 2012). 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 mobile phase refers to the liquid moving over the particles, and the stationary phase refers to a thin coating formed on the surface of small particles. Each component in a sample has a particular distribution equilibrium depending on its solubility in the phases and/or molecular size 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 shown in Figure 2.

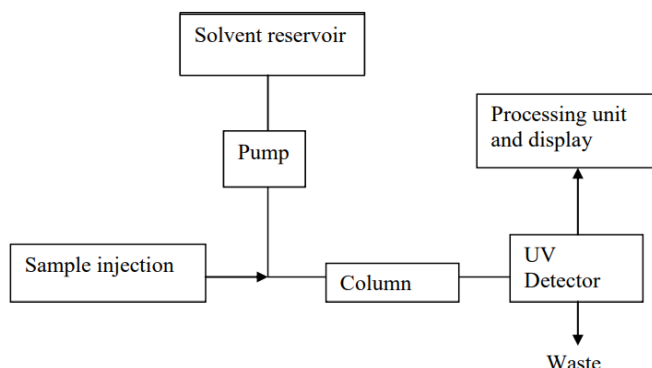


Figure 2: HPLC apparatus with UV detector represented in a diagram

2. MATERIALS AND METHODS

2.1. SAMPLE SITES AND SAMPLE COLLECTION

Twenty-eight samples of multi-floral fresh honey of *Apis Mellifera 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. After collection, these samples were brought to the laboratory and were kept at temperature between 4 and 10°C until analysis. For the present study, the map of Eastern Province and sampling sites were prepared.

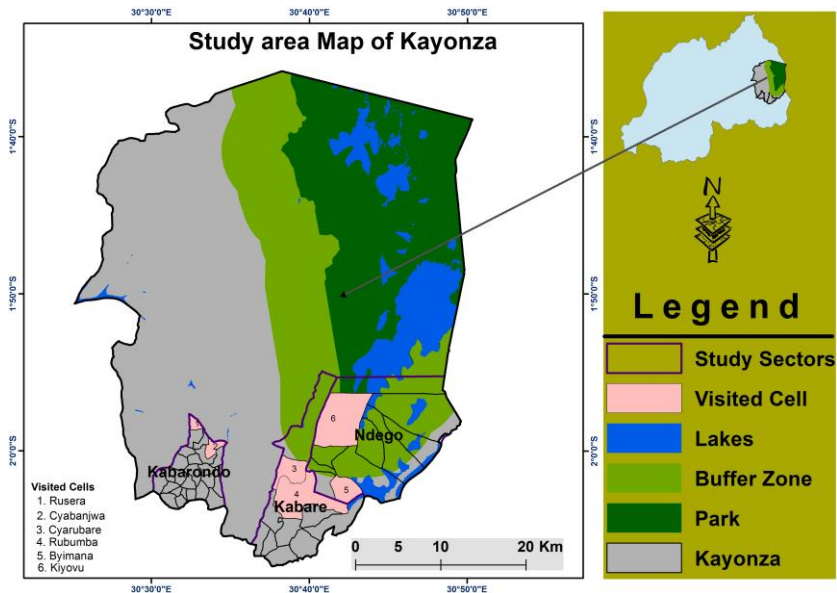


Figure 3: Study area at Kayonza District

2.2. Chemical reagents, solvents and standards

Pesticide analytical standards that were used and their purity is given; Abamectin (95.5%), Alpha-cypermethrin (99.0%), Chlorothalonil (99.7%), Metalaxyl (99.1%), Profenofos (99.4%) and Deltamethrin (98.6%). Acetonitrile HPLC grade, distilled water-HPLC grade, ethyl acetate, anhydrous sodium sulphate, C18-bonded silica gel (50 µm) were also used.

2.3. Extraction and clean-up of samples

For the extraction of pesticide residues, the method of Farooqi et al., (2015) was employed with a few modifications.

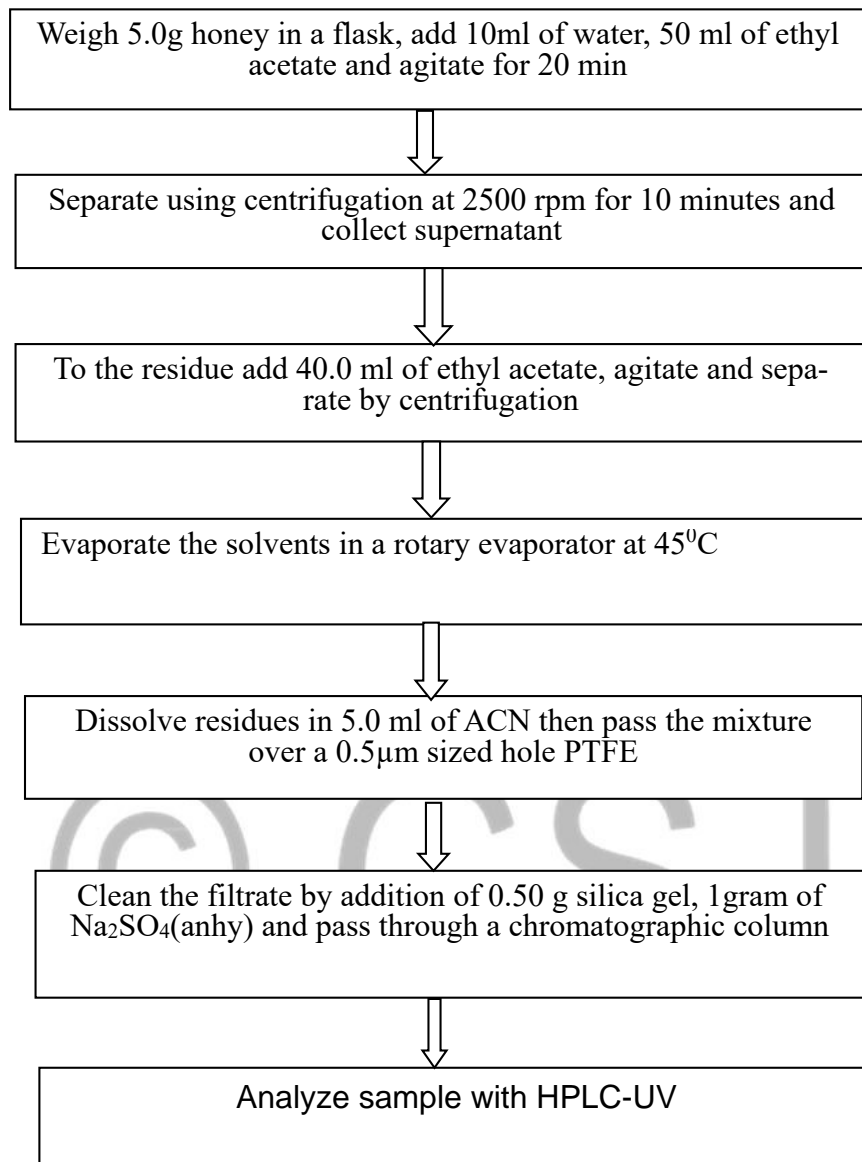


Figure 4: Scheme of the extraction process

2.4. HPLC-UV instrumentation and operating conditions

Pesticide residues in honey were evaluated using a High-performance Liquid Chromatography-UV detector system (Al-rimawi, 2014). The system of Shimadzu HPLC-UV consisted of a degassing unit (DGU20A₅), 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°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 (Al-rimawi, 2014; Farooqi et al., 2015). These instrumental conditions are chromatographic separation parameters.

2.5. Method validation

A method validation is a procedure of defining an analytical requirement and approving that the method under consideration has performance abilities reliable with what the application needs (Bernal, 2014). Analysis reliability is ensured via method validation. The variables precision, accuracy, linearity, and limits of detection (LOD) and quantification (LOQ) were all taken into accounts 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 the accuracy of the data acquired is between 70 and 110% with relative standard deviations (RSDs) not exceeding 20% (Farooqi et al., 2015). Prior to the analysis of samples, this was carried out daily. Different known concentrations (0.001, 0.01, 1, 10 and 50 $\mu\text{g}/\text{mL}$) 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.

2.6. Identification and quantification

By comparing the retention times of the sample peaks with those of the standard peaks and the amount of residue recorded in the integrator chart, the chemical compounds that make the pesticide residues were evaluated. The formula used to determine the amount of pesticide residue in $\mu\text{g}/\text{g}$ (ppm) is as follows:

$$\text{Pesticide residues in ppm} = \frac{A_{sa} \cdot C_s \cdot V}{A_s \cdot W}$$

Where, A_{sa} : Sample peak area; A_s : standard peak area; C_s : standard concentration, $\mu\text{g}/\text{ml}$ W : Sample weight in grams and V : Sample volume overall in ml.

3. RESULTS AND DISCUSSIONS

3.1. Linearity and range

Calibration curves using pesticide standards were created to determine where a pesticide maintains a linear response between the amount of analyte and the response from the HPLC-UV. Considering the Food and Drug Administration Office of Regulator's validation process (FDA, 2020), the linearity of the method is reflected as satisfying when R^2 is between 0.96-1.0. According to the Food and Drug Administration's validation principle, the R^2 values (Table 5) are helpful for quantification. These values maintain the results of the recovery experiments 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.

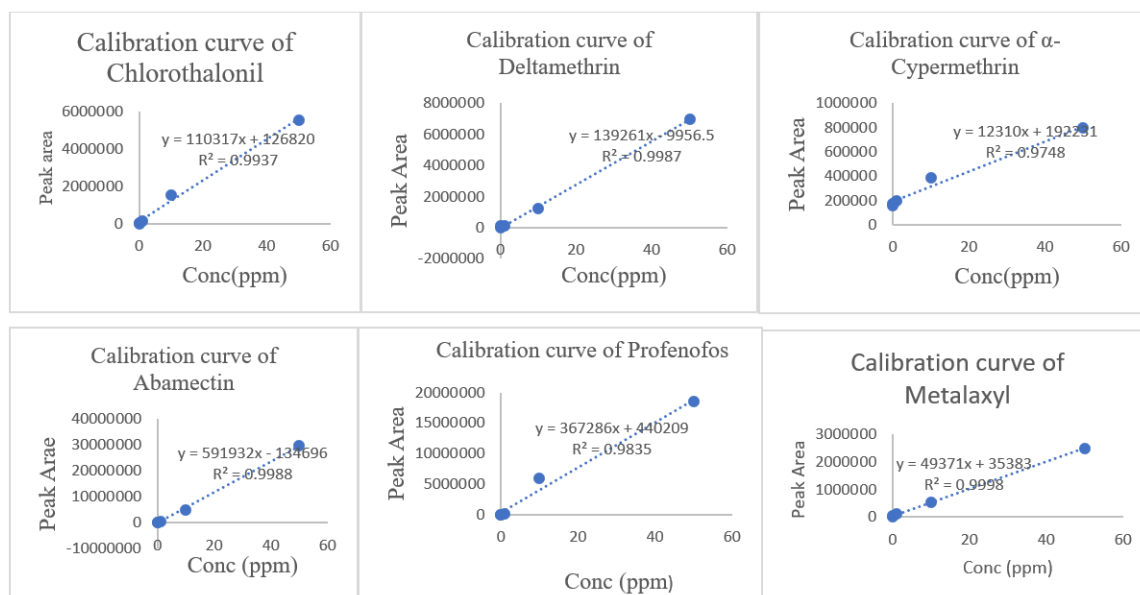


Figure 5: Calibration curves

3.2. Limit of quantification (LOQ) and Limit of Detection (LOD)

The lowest concentration providing a response that is three times the baseline noise determined from the analysis of the control (untreated) sample was determined to be the limit of detection (LOD, mg/L). The lowest concentration of a specific pesticide that produces a reaction that is 10 times the background noise was determined to be the limit of quantification (LOQ, mg/L)(Bernal, 2014).

Table 1: LOD and LOQ in mg/L for analyzed pesticides

Pesticide	LOD	LOQ	Equation	Linearity (R ²)	P-value
Alpha 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	0.046	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

LOD and LOQ are expressed as ppm (mg/L) of solution; R², linearity greater than 0.97, and P-value less than 0.05 indicate statistical significance of the findings between variable and response, therefore, according to (European Commission, 2021), R² 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.

3.3. 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 0.5 mg/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%.

Table 2: 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.

3.4. Results of analyzed pesticides

Table 3 shows the results of analyzed pesticides in 28 honey samples of *Apis Mellifera L.* by HPLC-UV. For 28 samples, only 5 samples contain very small amount(traces) of pesticide residues. The mean concentration of residues was ranged between 0.024-0.048mg/kg while the residues of deltamethrin are ranged between 0.014-0.015mg/kg. Residues of Profenofos, Alpha-cypermethrin, Chlorothalonil, and Metalaxyl were not detected at any sample of honey collected. All detected residues of pesticides were below maximum Residue limit(MRLs) (European Commission, 2021). In the different programs, numerous experts have found evidence of the contamination of honey from various parts of the world with various pesticide residues.

Table 3: Pesticide residues detected in honey (mg/kg) of *Apis Mellifera L.* from Kayonza District

Pesticide	Abamectin	α -Cyperme- thrin	Chlorothalonil	Metalaxyl	Deltamethrin	Profenofos
MRL	0.05	0.05	0.05	0.05	0.03	0.01
Kabare 1	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabare 2	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabare 3	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabare 4	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabare 5	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabare 6	BLOD	BLOD	BLOD	BLOD	0.015 ± 0.001	BLOD
Kabare 7	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabare 8	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabare 9	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD

Kabare 10	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Ndego 1	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Ndego 2	BLOD	BLOD	BLOD	BLOD	0.014 ± 0.003	BLOD
Ndego 3	0.048 ± 0.002	BLOD	BLOD	BLOD	BLOD	BLOD
Ndego 4	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Ndego 5	0.034 ± 0.003	BLOD	BLOD	BLOD	BLOD	BLOD
Ndego 6	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Ndego 7	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Ndego 8	0.024 ± 0.001	BLOD	BLOD	BLOD	BLOD	BLOD
Ndego 9	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Ndego 10	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabarondo 1	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabarondo 2	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabarondo 3	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabarondo 4	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabarondo 5	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabarondo 6	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabarondo 7	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD
Kabarondo 8	BLOD	BLOD	BLOD	BLOD	BLOD	BLOD

Results are stated as means ± standard deviation, and whereas, **BLOD** stands for **Below Limit of Detection**. The results obtained show that traces of abamectin pesticide are 0.048; 0.034 and 0.024 mg/kg in Ndego Sector, traces of deltamethrin are 0.015mg/kg, 0.014mg/kg in Kabare and Ndego sectors respectively. The results obtained show that no sample of honey contained pesticide residues greater than Maximum Residue limit as published by (European Commission, 2021).

In the 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 the Ndego Sector. In the Ndego Sector, there is a plantation of about 650 hectares in the Ndego Sector. This is due to the large practice of pesticides in this region dedicated to a maize crop. 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, and farms in the Ndego sector, Kayonza District. And also, the location of honey bees is close to the farm activities which is the cause of pesticide residues in honey.

The results obtained show that one sample of honey had residues of pesticides in Kabare sector. This is to the minimum practice of pesticides in this region.

In the Kabarondo sector and also in other sectors in the same region, like Nyamirama, and 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 detected in samples of honey that were gathered from the Kabarondo Sector due to the low practice of pesticides in their non-

developed farming in this region.

3.7. Assessment of 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 (Raghunandan, 2013). *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 samples of honey were polluted with pesticide residues, but less than the maximum residue limits. 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 the application of pesticides to combat worms, Sucking pests, beetles, aphids, and spider mites.

The honey samples from the Ndego sector contain traces of pesticide residues in a range between 0.014 and 0.048 mg/Kg and were below the MRLs of abamectin and deltamethrin, 0.05 and 0.03mg/Kg, respectively (Table 2) according to EU food safety (Commission, n.d.) and have no effect on any biological things or humans.

The findings of different researchers from the biosphere (Bogdanov, 2006; Eissa et al., 2014; Ben Mukiibi et al., 2021) agree with the outcomes of current research since there have been numerous reports of pesticide residues in honey products but the contents can be different due to the use of different techniques for analysis and sample collection from different sources.

4. CONCLUSIONS AND RECOMMENDATIONS

From the results of the study, honey from Kayonza District was free from pesticide residues. Very few samples have traces of the considered pesticides. The residues of abamectin were detected in three (3) honey samples 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% 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(Commission regulation, 2006). 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 BLOD to 0.048 mg/kg for abamectin and from BLOD to 0.015 mg/kg for Deltamethrin. Honey with these results is under the fulfillment requirement on pesticide residues on the market. Therefore, it can be concluded that the honey from Kayonza is safe for consumers. The residues of profenofos, Chlorothalonil, Metalaxyl, and Alpha-cypermethrin were not perceived in any honey sample from the Kayonza District.

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:

- (i) There is a need for periodic quality control mechanisms of pesticide residues in food products using laboratory equipment like GC-MS and HPLC-MS.
- (ii) 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. Monitoring campaigns should be extended to many sources: farms, markets, raw honey, processed honey, etc.
- (iii) Reducing the use of pesticides for the duration of blooming phases of plant life and not spraying when the wind is blowing

Acknowledgment

The authors wish to thank Beekeepers cooperatives in Kayonza District for their support in honey sampling at Kayonza District.

References

- Al-rimawi, F. (2014). a Hplc-Uv Method for Deteermination of a Hplc-Uv Method for Deteermination of Three Pesticides. *International Journal of Advances in Chemistry (IJAC)*, 2(August), 1–8.
- Alehagen, M. (2011). Development of a method for determination of pesticide residues in honey using liquid chromatography tandem mass spectrometry. *Master Thesis*, 1(1), 1–42.
- Alghamdi, B. A., Alshumrani, E. S., Saeed, M. S. Bin, Rawas, G. M., Alharthi, N. T., Baeshen, M. N., Helmi, N. M., Alam, M. Z., & Suhail, M. (2020). Analysis of sugar composition and pesticides using HPLC and GC–MS techniques in honey samples collected from Saudi Arabian markets. *Saudi Journal of Biological Sciences*, 27(12), 3720–3726. <https://doi.org/10.1016/j.sjbs.2020.08.018>
- Aryal, K. K., Neupane, S., Lohani, G. R., Jors, E., Neupane, D., Khanal, P. R., Jha, B. K., Dhital, M., Shrestha, B. M., Bista, B., Poudyal, A., & Karki, K. B. (2016). Health Effects of Pesticide among Vegetable Farmers and the Adaptation Level of Integrated Pest Management Program in Nepal , 2014. *Nepal HealthResearch Council, Governmnet of Nepal, April 2017*, 1–47. <http://nhrc.gov.np/>
- Barganska, Z. (2014). Determination of Pesticide Residues in Honey using the GC×GC-TOFMS Technique. *Journal of Bioprocessing & Biotechniques*, 04(07). <https://doi.org/10.4172/2155-9821.1000182>
- Ben Mukiibi, S., Nyanzi, S. A., Kwetegyeka, J., Olisah, C., Taiwo, A. M., Mubiru, E., Tebandeke, E., Matovu, H., Odongo, S., Abayi, J. J. M., Ngeno, E. C., Sillanpää, M., & Ssebugere, P. (2021). Organochlorine pesticide residues in Uganda’s honey as a bioindicator of environmental contamination and reproductive health implications to consumers. *Ecotoxicology and Environmental Safety*, 214. <https://doi.org/10.1016/j.ecoenv.2021.112094>
- Bernal, E. (2014). Advances in Gas Chromatography. *Advances in Gas Chromatography, February 2014*. <https://doi.org/10.5772/57016>
- Bogdanov, S. (2006). Contaminants of bee products. In *Apidologie* (Vol. 37, Issue 1, pp. 1–18). <https://doi.org/10.1051/apido:2005043>
- Commission regulation. (2006). REGULATION (EC) No 396/2005 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. *Journal of the European Communities*, 1881(February 1998), 1–5. <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2006R1881:20100701:EN:PDF>
- Eissa, F., El-sawi, S., & Zidan, N. E. (2014). Determining Pesticide. *Journal of Bioprocessing & Biotechniques*, 23(5), 1573–1580.
- European Commission. (2021). Guidance document on analytical quality control and method validation for pesticide residues analysis in food and feed SANTE 11312/2021. *Sante/11312/2021*, 1–57. https://ec.europa.eu/food/system/files/2022-02/pesticides_mrl_guidelines_wrkdoc_2021-11312.pdf
- Farooqi, M. A. (2015). I dedicate this humble effort to Great scientific reformer Holy Prophet (P . B . U . H). *Thesis*, 1–138.
- Farooqi, M. A., Ul-Hasan, M., Sabri, M. A., & Javed, N. (2015). Assessment of insecticide residues in raw honey by high performance liquid chromatography with ultraviolet detection. *Pakistan Journal of Zoology*, 47(4), 965–970.
- FDA. (2020). Methods, Method Verification and Validation. *Food and Drug Administration Office of Regulatory Affairs, II*, 1–32.
- Garibaldi, L. A., Carella, D. S. G., Jodar, D. N. N., Smith, M. R., Timberlake, T. P., & Myers, S. S. (2022). Exploring connections between pollinator health and human health. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 377(1853). <https://doi.org/10.1098/rstb.2021.0158>
- Hamilton, D. (2004). Pesticide residues in food—acute dietary exposure. *Pest Management Science*, 311–339. <https://onlinelibrary.wiley.com/doi/full/10.1002/ps.865>
- Houbraken, M., Habimana, V., Senaev, D., López-Dávila, E., & Spanoghe, P. (2017). Multi-residue determination and ecological risk assessment of pesticides in the lakes of Rwanda. *Science of the Total Environment*, 576, 888–894. <https://doi.org/10.1016/j.scitotenv.2016.10.127>
- Irungu, J., Raina, S., & Torto, B. (2016). Determination of pesticide residues in honey: A preliminary study from two of Africa’s largest honey producers. *International Journal of Food Contamination*, 3(1). <https://doi.org/10.1186/s40550-016-0036-4>
- Lasheras, R. J., Lázaro, R., Burillo, J. C., & Bayarri, S. (2021). Occurrence of pesticide residues in Spanish honey measured by QuEChERS method followed by liquid and gas chromatography–Tandem mass spectrometry. *Journal Article*, 10(10). <https://doi.org/10.3390/foods10102262>
- Nazir, S., N, R., & K, A. (2017). Comparative Evaluation of Extraction Procedures and Chromatographic Techniques for Analysis of Multiresidue Pesticides in Honey. *Journal of Environmental and Toxicological Studies*, 1(1). <https://doi.org/10.16966/2576-6430.102>
- NISR. (2012). *EICV3 Thematic report: Agriculture* (Issue August).
- Oyugi, R. B. (2012). *Pesticide Residues in Some Vegetables Rotated With Tobacco Using Hplc, and Farmers’ Awareness of Pesticide Health Effects in Kuria-Migori, Kenya. November*, 1–124.
- Priyanka, B. (2020). *Quality Parameters Determining the Purity of Honey*. Bee Hively Sustainable and Organic.
- Rundlöf, M., Nilsson, H., & Smith, H. G. (2008). Interacting effects of farming practice and landscape context on bumble bees. *Biological Conservation*, 141(2), 417–426. <https://doi.org/10.1016/j.biocon.2007.10.011>
- Sanchez-Bayo, F., & Goka, K. (2016). Impacts of Pesticides on Honey Bees. *Beekeeping and Bee Conservation - Advances in Research*. <https://doi.org/10.5772/62487>
- Sary, D. N. (2018). Introduction to High Performance. Liquid Chromatography. *FRAKSINASI KROMATOGRAFI KOLOM METABOLIT SEKUNDER*, 5(September), 188–194.
- Siebers, J., Binner, R., & Wittich, K. P. (2003). Investigation on downwind short-range transport of pesticides after application in agricultural crops. *Chemosphere*, 51(5), 397–407. [https://doi.org/10.1016/S0045-6535\(02\)00820-2](https://doi.org/10.1016/S0045-6535(02)00820-2)

- Tarek, E., Abdel-Rahman, A., Eissa, A., & Mahfouz, M. (2014). Assessment of pesticide residues in honey and their prospective risk to consumers in Article History. *Journal Article*. www.ejppri.eg.net
- Wang, F., Wang, Y., Li, Y., Zhang, S., Shi, P., Li-Byarlay, H., & Luo, S. (2022). Pesticide residues in beebread and honey in *Apis cerana cerana* and their hazards to honey bees and human. *Ecotoxicology and Environmental Safety*, 238(December 2021), 113574. <https://doi.org/10.1016/j.ecoenv.2022.113574>
- WHO and FAO. (2014). The international code of conduct on pesticide management : guidelines on highly hazardous pesticides. *Report*, 35. www.fao.org/publications

© GSJ