UNIVERSITY of RWANDA

COLLEGE OF SCIENCE AND TECHNOLOGY

SCHOOL OF SCIENCE

Assessment of the presence of Abamectin, Cypermethrin, Cyhalothrin, Profenofos residues in tomatoes, cabbages and oranges grown in Eastern province, Rwanda.

A dissertation submitted in partial fulfillment of the requirements for the Degree of Master of Science (MSc) in Environmental Chemistry

By

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DECLARATION

Student:

I, Emmanuel NKURUNZIZA, declare that this dissertation titled "assessment of the presence of Abamectin, Cyhalothrin, Cypermethrin and Profenofos residues in tomatoes, cabbages and oranges grown in Eastern province, Rwanda" is the result of my own work and has not been submitted for any other degree at the University of Rwanda or any other institution.

Signature:

Main supervisor:

I, Prof. Theoneste Muhizi, the main supervisor of this dissertation titled" assessment of the presence of Abamectin, Cyhalothrin, Cypermethrin and Profenofos residues in tomatoes, cabbages and oranges grown in Eastern province, Rwanda" declare the approval submission of the dissertation for examination.

Signature:…………………………………..

DEDICATION

To

My Mother

My beloved wife and daughters

My brothers and sisters

with love and respect

ACKNOWLEDGEMENT

All praises be to God almighty, the most merciful, the omnipotent, the omnipresent who blessed and protected me until the successful completion of this study.

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ABSTRACT

The use of pesticides in agriculture is taken as alternative for increasing crops production and thus having sufficient food for population. However, continuous application of these chemicals has increasingly generated negative effects to the environment including farmers' diseases. Due to its high quantity in surface water allowing easy irrigation, the Eastern province of Rwanda is taken as garret for the production of fruits and vegetables. To protect these crops against various pests and to increase productivity, farmers in this region apply different types of pesticides using one way or another, depending on either their financial capacity or their intellectual skills and the risks for their intoxication and food contamination. This study aimed at knowing types of pesticides used in Eastern province of Rwanda, assessing their presence in different crops and advising consumers and policymakers. Types of these pesticides were known through a survey conducted to farmers and pesticides sellers, while chemical analysis from tomatoes, cabbages and orange was realized using High Performance Liquid Chromatography. Results from the survey realized on 272 farmers and 21 pesticides sellers indicated that Cypermethrin, Profenofos and Abamectin are the most applied insecticides while Mancozeb and Metalaxyl were the most fungicides used in this province. The method used by farmers to apply these chemicals indicated different illegal ways leading to the risk of human intoxication. Lack of trainings, mismanagement of agrochemicals, lack of personal protective equipment and appropriate spraying materials, are ones of the remarked issues. Yet, results from chemical analysis of pesticides residues showed that 23%, 13% and 12% of samples of tomatoes, cabbages and oranges, respectively are contaminated by pesticide residues. The most detected pesticide in tomato samples was Abamectin (1.25 ppm) followed by Cypermethrin (0.80 ppm), while the higher concentration of Profenofos was detected in both orange and cabbage with values of 1.19 and 1.06 ppm, respectively. Abamectin was detected in both orange and cabbage at concentrations of 1.10 and 0.85 ppm, respectively. Cypermetrin, Abamectin and Profenofos were detected in all analyzed samples of tomato, cabbage and orange. From this study, it was remarked that improvement of awareness on pesticides usage is highly needed for farmers, pesticides dealers and crops consumers to avoid possible risk of health intoxication. The analysis of the pesticides residues in all treated crops, soils, water and biological samples from this region are recommended for future studies.

Key words: Farmers, pesticides, Abamectin, Cypermethrin, Cyhalothrin, Profenofos, HPLC,

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LIST OF ABREVIATIONS

ABM: Abamectin

ACN: Acetonitrile

ADI: Accepted daily intake

ARfD: Acceptable reference dose

ANOVA: Analysis of Variance

BLOQ: Below limit of quantification

BUG: Bugesera

CBG: Cabbage

DDT: Dichloro-Diphenyl-Trichloro Ethane

EC: Emulsifiable Concentrate

EDI: Estimated daily intake

EDTA: Ethylene diamine tetra acetic acid

EU: Europian Union

GABA: Gamma Amino butyric acid

GPS: Global positioning system

HPLC: High Performance Liquid Chromatography

HPLC-RP: High Performance Liquid Chromatography-Reverse Phase

ICH: International conference of harmonization

IPCS: International Programme on Chemical Safety

KAY: Kayonza

LOD: Limit of detection

LOQ: Limit of quantification

MRLs: Maximum Residue Levels

NYAG: Nyagatare

ORG: Orange

PAN: Pesticide action network

PHI: Pre- harvest interval

PIC: Prior informed consent

PPE: Personal protective equipment

PPM: Part per million

REI: Restricted entry interval

S: Slope

Sd: Standard

SAICM: Strategic Approach to International Chemical Management

SSR: Spiked sample result

TMT: Tomato

USR: Unspiked sample result

ICCM: International conference on chemical management

SC: Suspension concentrate

SP: Soluble powder

UK: United Kingdom

USD: United State Dollar

UV: Ultra-violet

WDG: Water dispersible granule

WHO: World health organization

WP: Wettable powders

Chapter 1: GENERAL INTRODUCTION

1.1. Background

Rwanda like other developing countries is experiencing of an alarm population increasing with an annual population growth of 2.6 and population density of 525/km^2 [1, 2]. Agriculture is one of the priorities field of investment in order to achieve vision 2050, in parallel with environmental protection and sustainability [1, 3]. However, the sector is facing with different challenges. The increase in the country population could not have been possible without a parallel increase in food production leading to overexploitation of available land accompanied by agricultural malpractices [3, 4]. Due to the rapid urbanization, the arable soil for agriculture in Rwanda decreases at an alarm rate conducting to the declining of soil fertility [5, 6]. The increase of pest diseases is another challenge for farmers. To overcome the mentioned challenges, there is a need to increase soil productivity by using agricultural inputs like fertilizers in order to increase harvest on surface area and pesticides to manage pests and thus crop diseases. However, this measure is also increasing the risk of environmental contamination and thus health risks through indirect consumption of agrochemicals residues and direct exposure during application. The use of pesticides in Rwanda is continuously increasing [7]. This increasing application need special attention for environmental protection and reducing health risk effect [8].

Pesticides are group of chemicals applied in the environment with the aim to suppress plant and animal pests, protect agricultural and industrial products [9, 10]. Around 3 billion kg per year of agrochemicals are consumed globally with a budget of ~40 billion USD [11]. There is no doubt about the utility of these chemicals in improving crops productivity. Without pesticides application, there would be a loss of 78%, 54% and 32% production of fruits, vegetables and cereals respectively [4, 12]. However, the integration pest management should be a target for each user to avoid the excessive accumulation of pesticides in food chain and to protect the health of workers. In this regard, this study was conducted to assess the way pesticides are used and managed in agriculture and the level of residues in some fruits and vegetables consumed in Eastern Province of Rwanda and thus to advice both policy makers, farmers and consumers.

1.2. Problem Statement

Pesticides are toxic and deliberately spread in the environment and negatively affect non-target species. Consuming residues and metabolites of pesticides in food products has negative effects on human life [13]. Multiple studies have showed that using pesticides can be the source of many health effects like headache, nausea, skin allergy and may influence the progression of the disease [14]. In

Nyagatare around 90% of rice farmers had experienced of many health effects after applying pesticides [15]. Around 15% of applied pesticides in agriculture by Rwanda farmers are highly hazardous pesticides and these chemicals are banned in European union market [16]. Most applied agrochemicals like; Cypermethrin, Profenofos, Cyhalothrin and Abamectin are classified as highly hazardous pesticides by Pesticide action network (PAN) international due to the serious problems they caused to humans and biodiversity [17, 18].

In addition to this, the countries around Rwanda may use the pesticides which are not accepted in Rwanda, and this may lead to their fraudulent sold on Rwanda market [16, 19, 20]. In Rwanda, there are also limited data on pesticide residues status due to the lack of advanced analytical facilities [16, 21]. In addition, as its population is highly increasing in both rural and urban areas, the rate of consumption for both fresh vegetables and fruits is also increasing with domination of tomatoes and cabbages occupying percentages of 28.4% and 12.8%, respectively [22]. These two products are often consumed freshly without any other preparation. If they contain pesticide residues they may lead to high pesticides exposure [2, 16, 23–25]. It is therefore necessary to assess the levels of pesticides in some food products consumed in Rwanda.

1.3. Research Justification

The study was conducted in Eastern Province of Rwanda due to different reasons. The site for survey, Eastern Province, is the biggest with more agricultural land, warmest, driest province in Rwanda (temperature 22-25^{o}C) and the most producer of fruits and vegetable [24, 26]. In fact, during the year 2013, 69% and 32% of the produced fruits and vegetables, respectively were coming from this Eastern Province of Rwanda [24]. The more the weather is warm, the more the types of pest increase and thus the quantity of pesticides used [27]. In addition, the quantity of pesticides used at region with high temperature is relatively high compared to those used in the region with low temperature because of the risk difference in evaporation [28, 29]. The production, distribution, and use of pesticides require strict regulation and control due to their toxic effects [30]. An assessment of pesticides products, their application and farmers perceptive is very important in order to protect the health of workers and to avoid excessive residues on consumed food. This monitoring is also important to farmers on their profitability. Horticulture farming has the potential of trading at international level [2, 23, 25, 31]. In order to be accepted and sold at high price on international market, farmers have to make sure that the crops are free from excessive pesticides residues and this will be achieved through applying pesticide regulations, increasing of population awareness on these products and continuous monitoring [24, 25]. An assessment of health negative effects from these products on farmers and consumers is necessary for stakeholders and policy makers in order to plan for the way of their phase out and search for alternatives. The present study will provide a better understanding of pesticide benefits and probable negative effects on farmers and consumers. The knowledge generated in this research project will be essential to decision makers for setting or improving national pesticide policies and advanced strategies of their managements. The results generated from this research project on pesticide residues will be a baseline on further researches. The dissemination of the findings of this research project through the national, regional and international seminars, publications and conferences will increase the awareness on the harmful of pesticides on the human life.

1.4. Research Objectives

1.4.1. General objective

The general objective of this study was to determine the level of pesticide residues in tomatoes, cabbages and oranges grown and consumed in Eastern province, Rwanda.

1.4.2. *Specific Objectives*

The specific objectives of this study were:

i. Conducting a survey on pesticide use in agriculture in Eastern Province of Rwanda
ii. Assessing the risk associated to the environmental pollution from pesticides application in eastern province Rwanda.
iii. Determining the level of Abamectin, Cypermethrin, Cyhalothrin, and Profenofos residues in tomatoes, cabbages and oranges consumed in Eastern province of Rwanda.

1.5. Research Questions

The general question of this study was “how pesticide application by eastern province Rwanda farmers’ can affects health of farmers, consumers and environment?”

This study aimed answering the following specific questions:

i. Which pesticides are used in agricultural in Eastern province of Rwanda?
ii. Is there any evidence showing that in Eastern province Rwanda, pesticide application contributes to the environmental pollution?
iii. What are the levels of Abamectin, Cyhalothrin, Cypermethrin and Profenofos residues in tomato, cabbage and orange which are consumed in Rwanda?
iv. Is there any risk from consuming tomatoes, cabbages and oranges grown in Eastern province of Rwanda?

1.6. Scope and Limitation

This study was limited both in time and in space; according to the necessary work and limited funds, the study has been conducted from December 2020 till December 2021 on 7 districts of Eastern province Rwanda. The focus was done on the following four insecticides Abamectin, Cyhalothrin, Cypermethrin and Profenofos in tomatoes, cabbages and oranges crops but their metabolites was not considered.

1.7. Research Outlines

This research work is divided into five main chapters with appendices. The first chapter presents the general introduction which focus on the background of the work, the problem statement and research justification, the objectives, the research questions, scope and limitations and finally the report outline to highlight the structure of the dissertation. The second chapter presents mainly the literature review which focuses on previous studies done on pesticide application. Classification, formulations and applications of pesticides, generalities on pesticides targeted in this research, environmental and health impact of pesticides and their management in Rwanda are highlighted in this chapter. The third chapter presents not only the methodology applied to carry out this study; but also instrumentation used and data analysis were also described. After that the forth chapter is the presentation and analysis of the results. Finally, conclusion and recommendations were formulated according to the objectives of the study and research findings.

Chapter 2: LITERATURE REVIEW

2.1. Classification of pesticides

Pesticides are natural or synthetic products used mainly in agriculture, in household or in industry to control insects, unwanted plants, rodents, spiders, fungus and nematodes. These products are classified based on different criteria such as active ingredients, chemical classes, their mode of action and type of pests or diseases to control [32].

2.1.1. *Classification based on type of pest*

According to Akashe et al. [33], pesticides groups can be classified referring to the type of pest organisms they are controlling. Thus, insecticides, fungicides, herbicides, acaricides, molluscides and rodenticides can be recognized among more others. Insecticides are controlling insects and contain chemicals like pyrethroids, organophosphorus, carbamates, organochlorine and manganese compounds. Beside, fungicides have been designed to kill and manage fungus and they contain thiocarbamates, dithiocarbamates, cupric salts, tiabendazoles, triazoles, dicarboximides, dinitrophenoles and organotin compounds. Herbicides are the products used to control unwanted plants. Among this group, Bipyridyls, Chlorophenoxy, Glyphosate, Acetanilides and Triazines are found. In addition, nematicides control plant-parasitic named nematodes, while acaricides are for fighting arachnid-mites. Furthermore, molluscides and rodenticides (Warfarines and Indanodiones) are used to control molluscs and rodents, respectively [33].

2.1.2. *Classification based on mode of action*

Behind classification based on the type of pest, pesticides can also be classified by their mode of action. Depending on the active components in the pesticides and the targeted sites of action, the way of inhibiting or eliminating the unwanted pests is greatly changing. Considering this assumption, different classes including neuromuscular toxins, insect growth regulators (IGRs) and gut disruptors can be distinguished among others. On one side, neuromuscular toxins include compounds like acetylcholinesterase and GABA-gated attacking the nervous system or muscles of pest by inhibiting nervous action. In paralyzing or malfunction of nervous system, pest cannot move properly and became weak. On other side, insect growth regulators (IGRs) affect the growth or development of pest by interacting with their metabolism. Gut disruptors are the products which can alter the gut microbiota composition by destroying the integrity of the gut lining [34, 35].

2.1.3. *Classification based on chemical composition*

Chemical composition is very important tool to classify chemical drugs and to indicate the main functional group they contain. Using this classification, organophosphates, organochlorines, synthetic pyrethroids, carbamates and dithiocarbamates can be taken as examples among others.

The first group, organophosphates pesticides is made by insecticides made by central phosphate molecule with substituents like alkyl group or aromatic. It includes Parathion, Profenofos, Diazinon, Chlorpyrifos, Malathion, Dimethoate and Acephate [32, 36]. This group of products is highly toxic to mammalian including human by affecting the function of the nervous system. The second group, organochlorines includes chlorinated hydrocarbons insecticides which break down slowly and some are very persistent in the environment [32, 37]. It includes pesticides like DDT, Methoxychlor, Lindane, Mirex, Chlordane, Dieldrin and Toxaphene [37]. Pyrethroids constitute the third group which contains organic compounds similar to pyrethrins and produced from pyrethrums [32]. They have low persistence and are moderate toxic to man. However, they are very toxic to aquatic organisms [38]. The group of pyrethroids includes Cyfluthrin, Permethrin, Deltamethrin, Cypermethrin, Bifenthrin and Cyhalothrin [32]. The fourth group among pesticides is carbamates. These are organic compounds insecticides derived from carbamic acid. When ingested in the organism, they are rapidly detoxified and eliminated from animal tissues [32]. They are reversible acetyl cholinesterase inhibitors with shorter duration action [32]. This group of compounds includes Carbaryl, Oxamyl, Carbofuran, Thiodicarb and Methomyl. The fifth group is composed by dithiocarbamates which are fungicides containing two groups of chemicals, dimethyldithiocarbamate and ethylenebisdithiocarbamate. Depending on metal cation which is present in chemical structure, their classification depends on these metals and introduces the names of maneb and zineb or ziram for the presence of manganese and zinc, respectively. These compounds have low acute oral and dermal toxicity. On the other hand, chronic exposure to dithiocarbamates leads to adverse effects due to contact with dithiocarbamate acid or metal ligand [39].

2.1.4. *Classification based on toxicity*

Toxicity of pesticides means their effect on non-targeted living things. The role of pesticides is to fight against unwanted organisms leaving safe other ecological components. However, as chemicals, when used, these products are negatively affecting environment including non-targeted organisms leading to different environmental hazards. In this way, five classes of toxicants symbolized by Ia, Ib, II, III, and IV are recognized depending on the rate of toxicity caused. The first class of extremely hazardous pesticides (Ia) includes those products with less than 5 mg/kg as LD_{50} for oral exposure.

Most of the products in this group are restricted by Rotterdam and Stockholm conventions. The chemicals like; Hexachlorobenzene, Mercuric chloride, Calcium cyanide are classified in this group. The second group of highly hazardous pesticides (Ib) contains the substances with LD_{50} between 5 and 50 mg/kg. The group contains the products like; Dichlorvos, Fluoroacetamide, Mercuric oxide, Sodium cyanide, Methiocarb, Tefluthrin [33, 40, 41]. The third group of moderately hazardous pesticide (II) contains the products with LD_{50} of oral exposure between 50 and 500 mg/kg [33, 40, 42]. Examples of chemicals classified in this group are; Bifenthrin, Butylamine, Chlorpyrifos, Copper sulfate, Deltamethrin, Dimethoate, Pyrethrins. The fourth group of slightly hazardous pesticides (III) is the group with LD_{50} between 500 and 5000 mg/kg and contains the products like; Acephate, Copper hydroxide, Amitraz, Hexazinone, Metalaxyl, Pyrifenox[33, 40, 41]. The last category is the unlikely to present acute hazard which characterized by LD_{50} more than 5000 mg/kg. Examples of products which are in this group are ; Amitrole, Biphenyl, Buprofezin, Captan, Cycloxydim, Dichlomezine, Dinitramine, Sulphur, Tetramethrin [43, 44].

2.2. Formulation of pesticides

Pesticide formulation is a combination of one or more active ingredients and several inert ingredients of chemical products which controls pests. The way in which active ingredients are formulated helps in their management by facilitating storage and safety handling. Different parameters of active ingredients like; solubility, toxicity and stability are treated and transformed through formulation with inert ingredients to allow pesticide efficiency [33, 45] These inert ingredients may lead to health risks of the end users. Different reason may guide in formulation selection: (i) the safety of applicator, (ii) environmental protection, (iii) the biology of the pest, (iv) availability of the equipment, (v) surface to be protected, and (vi) the cost [39, 46] The most common formulations can be solid or liquid type. Solid formulations may be concentrates (wettable powders, soluble powders and dry flowables), requiring dilution with water before being used, or ready-to use (pellets, granules and dusts), while liquid formulations can be liquid flowables made by mixing wettable powders before packaging, microencapsulates which are solid or liquid inert surrounded by a plastic or starch coating, emulsifiable concentrates which are composed by oil- active ingredient dissolved in an appropriate solvent, and solutions [33, 46]. When treating large areas, concentrated formulations are very useful due to the contents of high amount of active ingredient and easy calculation of dilution required. while for the ready to use formulations are adequate for small areas because of the limited amounts of active ingredients they contain[33, 45].

2. 3. Pesticide handling and storage

The management of pesticides including their storage, transportation, mixing, cleaning pesticide spills requires higher attention due to the severe toxicity they are manifested once ingested [47]. These products should be stored in a well ventilated sufficient space with a well-controlled temperature and humidity and in well lockable containers to avoid any unauthorized access. To avoid risk of environmental pollution and intoxication, stores of these products should be set away from water sources, animal feed and human food storage areas. During storage and usage, all instructions on labels should be seriously respected and applied as they are. The original containers are set in materials which are avoiding physico-chemical degradation of these products and therefore should be not exchanged before the pesticide active ingredients are finished [48]. Well trained people on the degradation and toxicity profile of these chemicals, are preferable in all activities related to handling, storage, mixing, spraying and restoring after usage. In addition, protecting equipment is a required condition to avoid any intoxication [47].

2. 4. Application of pesticides

In developing countries many challenges in the use of pesticides can be identified. These are for examples the lack of adequate equipment to be used in spraying these chemicals, insufficient training for farmers and the resistance of pests on the existing biocides. All these challenges can contribute on one way or another to intoxication of the users and thus to severe diseases. All agrochemicals like insecticides and fungicides are toxic to targeted and non-targeted living organisms. Users, dealers and producers of these chemicals are mostly exposed to intoxication through the activities of selling, storing, mixing, spraying, and cleaning used materials. Therefore the use of protecting equipment including boots, hats, gloves, sleeve shirts and chemical-resistant coveralls is very necessary to reduce risk of intoxication of the users of pesticides [28, 48, 49]. According to WHO, pesticide poisoning accounts for 300,000 deaths every year worldwide and these are mainly from occupational poisoning for people using frequently these chemicals in agricultural fields or being engaged in manufacturing industries [48]. As any other chemicals, trading of pesticides is more regulated to avoid any negative consequences they can generate when badly done. Industries watch over and emphasize on how the labels are designated according to the regulation. Labels should present clear instructions on dosage, application methods, warnings and safe disposal. The agrochemical dealers are the best source of required information related to the application and waste management and this require a basic knowledge on application and handling of these chemicals [48]. In addition, the way these chemicals are packaged should be well controlled and the contents of the containers, too [50]. After gathering these agro-chemicals, many technologies can be used by farmers in spraying activities. They are set to minimize all health risks which can be connected and to ensure that non target organisms are affected [50]. Furthermore, pesticide application technologies which are minimizing runoff or off-site movement are of interest. The way of avoiding a high environmental pollution, establishment of buffer zones between the application fields and watercourses, residential and/or built-up neighborhoods, as well as livestock and food storage areas is a necessary tool to think on by farmers before any spraying. According to Lorenz 2009 and Murema 1999, conditions in which fields equipment are used and calibrated in order to apply the correct dosage is another crucial point in pesticides spraying [48, 50] . Weather condition during spraying activities also may greatly influence the efficacy of the sprayed pesticides. Wet weather and windy conditions have to be avoided in the preference of warm weather and sunny journeys. A no respect of the usage protocols including required concentration and volume of the pesticides to be sprayed seriously affects the needed effect and thus leads to the decrease of the expected crops' production. In fact, bias in the use of pesticides may increase pest resistance and/or kill soil microorganisms leading to the reduction of the soil fertility and poor sustainability of agricultural production [48, 49] .

The entry of any person and animal in the sprayed area is not allowed during restricted-entry interval (REI) which may reach several hours. This time is set immediately after a pesticide application and is very necessary to protect human and animals against contamination. When two or more pesticides, with different restricted entry interval, are applied at the same time, the one of the longer restricted interval must take priority in the determination of the no entry time [48]. Furthermore, the management of the unused dilute pesticides, means not applied on the crops, empty pesticides containers and washings used in the pesticide containers has to be considered. All these wastes should be regulated by rules about the management of hazardous wastes [48]. Pesticides containers and obsolete pesticides have to be stored safely and securely prior to safe disposal. Their usage for other purposes are not allowed [48, 50].

2.5. Environmental and health impact of pesticides

The use and disposal of pesticides are done according to legislation and norms to minimize environmental contamination. During these activities, if badly realized, high amount of these products enter into the environment and the processes of their transfer, transformation and degradation by physicochemical parameters, sun light and microbes occur and contaminating other ecological components [10, 12, 50]. Depending on their chemical characteristics and different conditions of applied environment, degradations may occur from short time (hours) to the long time (months or years) and these define persistence characteristic of the concerned pesticide in environmental media and provide the concept of its half-life. During pesticide spraying, only a small amount of these products reach the target organisms and the remaining quantity reaches the soil and then water through erosion. In soil, pesticides are attracting with soil chemical particles, bound to them and seriously modify its normal structure and affecting its flora and fauna [12]. Once reaches water, aquatic biodiversity is affected and this contamination risks to be transmitted to human and other biota through food chain [51]. Worldwide, large amounts of pesticides are applied on soil and they are likely to leach or reach? surface and ground water and be the source of water pollution. Solubility and volatilization of pesticides are important factors determining the leaching rate and migration of these chemicals. In fact, pesticides dissolved in water can move from water to the soil and biota, while volatilization transforms their states, solid or liquid into gas, allowing them to reach atmosphere, to enter on air and be transported away from the treated surface [12]. Consequences of these contaminations have been shown by many studies on both human and animal health. Most acute toxicity from pesticide exposure reported are; dermatitis or inflation of the skin, nausea, vomiting, muscle weakness, diarrhea and headache [52]. Yet, the chronic effects also were reported like cancer effects, developmental effects, reproductive effects, the mutagenic effects and the hormone disruption [39, 41].

2.6. Generality on the concerned pesticides chemically assessed in this study

This study focused on the chemical analysis of Abamectin, Cyhalothrin, Cypermethrin and Profenofos. These chemicals occupy the second place for the usage in the Eastern Province after Mancozeb.

2.6.1. *Abamectin*

Abamectin is an insecticide used in agriculture to fight against insects like; ants, mites, and cockroaches. Abamectin product is a chemical belonging to the group of polycycliclactones (Figure 1). The product is produced biologically by fungus of actinomycete family, Streptomyces avermitilis [45, 53, 54]. Abamectin also can be synthetized chemically [55, 56].

It is an insecticide made by the mixture of two isomers; Avermectin B1a (>90%) and Avermectin B1b (< 10%). Abamectin is classified in Class II of the moderately hazardous [46–48]. This chemical has an acceptable daily intake of 0–0.001 mg/kg bw and an acceptable reference dose of 0.003 mg/kg bw [57, 58].

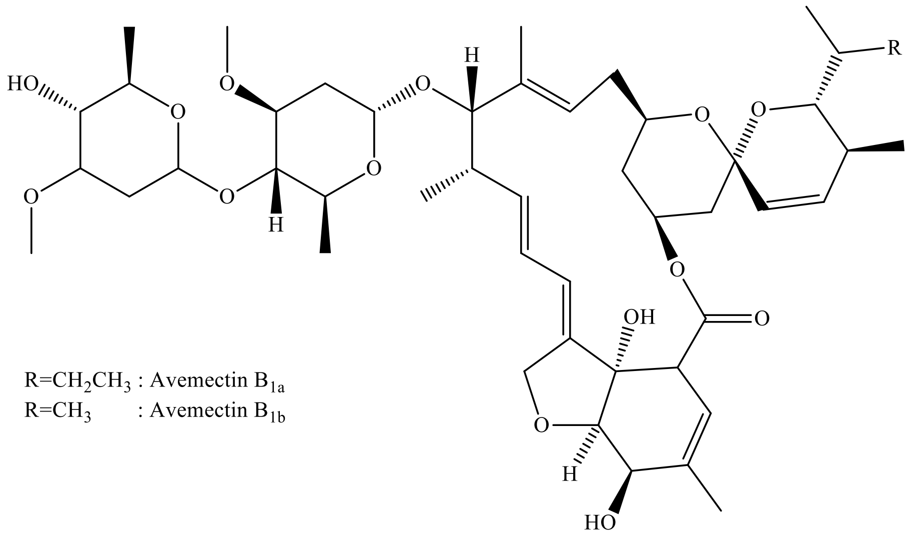
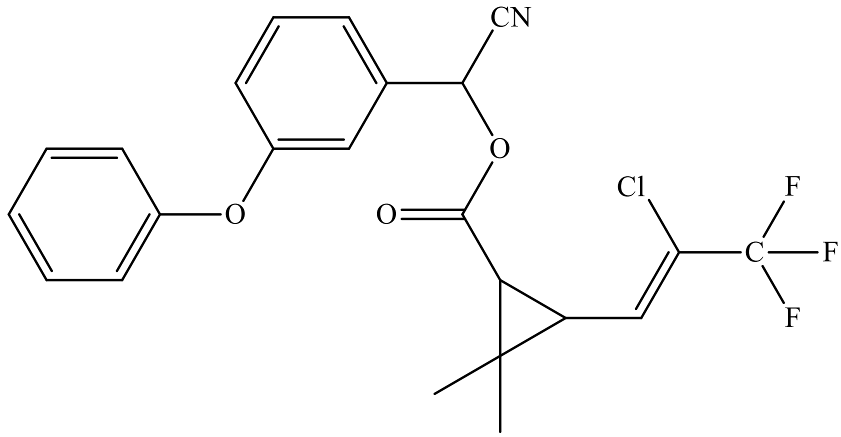


Figure 1: Abamectin chemical structure

The chemical is the nerve poisons and stimulates the gamma-aminobutyric acid (GABA) system inhibiting both nerve to nerve and nerve to muscle communication [59]. When treated, insect dies after becoming paralyzed in few days [45, 53, 54]. Abamectin is very toxic to aquatic living organisms like; fish, amphibians, crustaceans and wildlife. It is also highly toxic to beneficial insects including bees. The study showed that Abamectin is in the most insecticides affecting honeybees on their nerve and muscle cells and cause the faster death of bees [60]. Compared to the most insecticides, Abamectin was showed as faster in the death of honeybee workers [61]. On human, Abamectin may cause health effects like; nausea, vomiting, diarrhea, weakness, agitation in mild poisoning but severe poisoning causes hypotension, tachycardia, respiratory failure and coma. It is also harmful if absorbed through the skin [60].

2.6.2. *Cyhalothrin*

Cyhalothrin is one of the most applied insecticide used kill the insect pests like; ants, termites, cockroaches, spiders, earwigs, bees, bed, bugs, scorpions, silverfish, wasps, and ticks. Cyhalothrin is a pyrethroid insecticide (Figure 2). This group of chemicals is similar to the pyrethrins which are natural insecticides. Cyhalothrin has a low water solubility and it is nonvolatile.



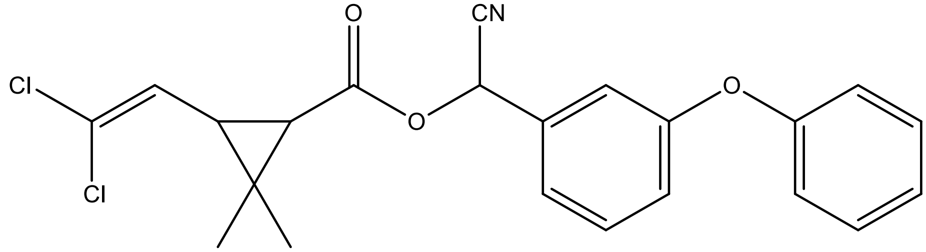
(*Z*)-cyano(3-phenoxyphenyl)methyl 3-(2-chloro-3,3,3-trifluoroprop-1-en-1-yl)-2,2-dimethylcyclopropanecarboxylate

Figure 2: Chemical structure of Cyhalothrin

Pyrethroids compounds disrupt the normal functioning of insect nervous system by delaying closure the sodium channel. Cyhalothrin affects neuromuscular system of target and non-target organism by disrupting the normal system [62]. After this disrupting, Cyhalothrin may influence insect paralysis or death. Like other pyrethroids, inhalation is the most exposure route of Cyhalothrin. The fish risk toxicity may be decreased by binding of this pesticide on soil and sediment. Cyhalothrin is highly toxic to bees [63].

2.6.3. *Cypermethrin*

Cypermethrin is in the most applied insecticide worldwide and it is used in many crops to kill pest insects like; ants, termites, cockroaches, spiders, earwigs, bees, bed, bugs, scorpions, silverfish, wasps, and ticks. Cypermethrin pesticide is one of the synthetic Pyrethroid insecticides, it is a synthetic chemical similar to the pyrethrum extract from the chrysanthemum plant family (figure 3). Cypermethrin like other pyrethroids synthetic insecticides is very effective than Pyrethrins (natural insecticides) [64–66].



cyano(3-phenoxyphenyl)methyl 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

Figure 3: Chemical structure of Cypermethrin

Cypermethrin like other Pyrethroids kills insects by the disrupting functioning of their nervous system mechanism. In animal system, nerve impulses travel along nerves when the nerves become momentarily permeable to sodium atoms and this permits sodium to flow into the nerve. This mechanism is the same to human and all animals. Cypermethrin and all pyrethroids delay the closing of the gate which allows the sodium flow [64, 65]. This insecticide also inhibits the aminobutyric acid receptor which causing excitability and convulsions. In addition, Cypermethrin inhibits calcium uptake by nerves, and at the same time it inhibits monoamine oxidase, an enzyme that breaks down neurotransmitters [65, 66].

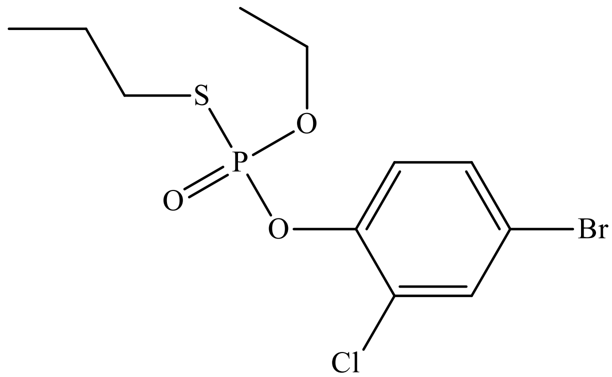
Cypermethrin has a high toxicity to aquatic living organisms like fish and insects. This toxicity also is very high to insects like bees, it kills insects come into contact with it by quickly affecting the insect's central nervous system. The toxicity of Cypermethrin on birds is very low [64, 65].

Cypermethrin exposure to human can cause different health effects like dizziness, nausea, headaches, burning, itching and seizures [42]. Cypermethrin also suppresses the immune system by inhibiting the formation of antibodies to disease-producing microbes. Cypermethrin and some Cypermethrin-containing products are skin sensitizers. This means that when Cypermethrin is applied to skin several times, later applications will have a more serious response than the first [39, 41, 64, 66].

In the body, esterase enzyme broke down Cypermethrin and other Pyrethroid insecticides. This enzyme is inhibited by organophosphate insecticides [42]. The combination of these two kinds of insecticides increase the toxicity because Cypermethrin will not be broken down at the same rate as it normally is. The result is that Cypermethrin and organophosphate insecticides are synergistic: the toxicity of Cypermethrin in combination with an organophosphate insecticide is greater than the toxicity of either insecticide alone [42].

2.6.4. *Profenofos*

Profenofos is one of the most commonly used organophosphate (figure 4) insecticides in the world. Emulsifiable concentrate (EC) of this product in various concentrations alone or in combination with Cypermethrin [67–69]. WHO in 2018 Recommended the current of acceptable daily intake 0–0.03 mg/kg bw and acceptable reference dose of 1 mg/kg bw [58].



O-(4-bromo-2-chlorophenyl) *O*-ethyl *S*-propyl phosphorothioate

Figure 4: Chemical structure of Profenofos

Like other organophosphates, the Profenofos mechanism of action is via the inhibition of the acetylcholinesterase enzyme. This pesticide is a neurotoxin acting as a cholinesterase inhibitor in insect nervous system [67–69]. It is the highest pesticides residues, moderately hazardous, toxic to human reproductive system by contributing to low sperm, reduced neurobehavioral performance and learning deficits [67–69].

2.7. Pesticides Management in Rwanda

2.7.1. *Agriculture Situation in Rwanda*

Rwanda has some limitation in agriculture field such as high population density which leads to the small plots for agriculture activities and many hills with limited area of flat land. The estimated arable land in Rwanda is 48% of the total area. These are the main limitations on profitability and productivity of Rwanda farmers. In addition, the education level of Rwanda farmers is also low. [31]. The government policy is to increase crops production through intensification of the high value crops which production needed on international markets. The focus is to maximize export revenues [3]. The cultivation of fruits, vegetables and flowers constitute horticulture farming and this type of agriculture is grown by more than half of rural households in Rwanda [70]. The high land reserved for horticulture in Rwanda is in Eastern province (figure 5). In 2013, this province was the most producer of orange in the country, the second producer of cabbage after southern province and second producer of tomato after western province [24]. In order to continue increasing foods productivity, the objective of Rwanda is to rely all farmers' activities to the policy of agriculture intensification by sometimes using chemicals and manures.

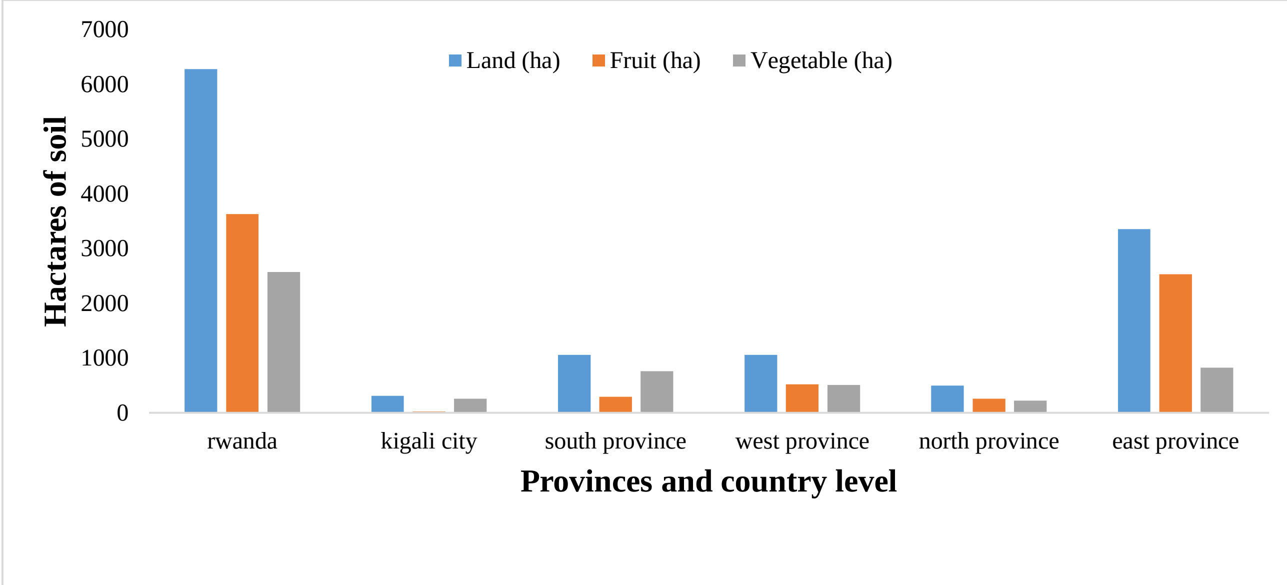


Figure 5: Land used for horticulture in Rwanda

2.7.2 ***Policy and regulations on the use of pesticides in Rwanda***

The laws regulating chemicals affecting living organisms are related to preventing and regulating environmental issues. Application of agrochemicals in farming is the main source of surface and groundwater pollution. In Rwanda, water borne diseases in households using chemical fertilizers and pesticides are increased from 11% and 24% in 2006 to 29% and 31% in 2011 respectively [16]. There is a challenge in managing the above pollution while simultaneously increasing agricultural harvest by transformation and intensification of farming. Several laws for the regulation of pesticides use have been developed and are currently implemented [71]. Particularly, in developing countries there are many difficulties associated with disposal due to the lack of proper facilities [72].

The vision of Rwanda on agriculture field is to become a nation that enjoys food security, nutritional health and sustainable agricultural growth from a productive, green and market-led agricultural sector [3, 31, 73]. In order to achieve this vision, some crops like maize, rice, potatoes, beans, banana, fruits and vegetables, have been selected to increase foods productivity [2, 3, 73, 74]. Furthermore, income from Rwanda's horticulture exports have been increased from 5 in 2005 to 25 million USD in 2018 [22]. The increasing of production in agriculture exports is associated to the rapid consumption of agriculture inputs. The investment in agriculture inputs has rapidly increased from 593 tons in 2001 to 6013 tons in 2019 and the import value from 1,803,200 USD in 2001 to 26,230.000USD in 2019 [7]. The increase of these agriculture values relied on changes remarked in agriculture practices including the use of chemicals to fight against pests. In order to achieve this target, the country is in the process of transforming subsistence farming into commercial agricultural and fully income generating [16]. Despite the established evidence of intensive use of pesticide in tomatoes, cabbages and fruits farming, there is limited information on pesticide residues in these crops as well as possible dietary exposure of pesticide from their fresh consumption [23, 74]. In order to protect consumers, different policies and regulations guiding the use and trade of these chemicals have been elaborated [71].

In the same line, pesticide policies in Rwanda are well developed, embedded in a consistent legal and institutional framework [71]. These policies are connected from Rwanda national constitution through international agreement of pesticides management and environmental protection policies. The aim of these policies is to manage pesticides from their registration to the disposal off of the obsolete pesticides and the empty containers [16]. For examples, not only the Ministerial order no 002/11.30 of 14/07/2016 determines the regulations governing registration of agrochemicals, but also the Republic of Rwanda has ratified different international conventions, including the Stockholm Convention on persistent organic pollutants, the Rotterdam convention on prior informed consent procedure for certain hazardous chemicals and pesticides in international trade, the Montreal protocol, strategic approach to international chemicals management which are related to the use of chemical products [16].

Besides these regulations, the pesticide industry in Rwanda is not yet well developed. Therefore, chemicals are mainly imported and this process is greatly controlled by the Government institutions including Rwanda FDA, RSB and RICA. The companies importing these chemicals are mainly Balton, Agrotech, Agropy, and ITG. Agro dealers are requested to be registered and their number in 2020 was estimated between 1000-2000 [16].

2.7.2. *Quantity and quality of the pesticides used in Rwanda*

The pesticide use in Rwanda is still limited to high income crops such as fruits, vegetables, potatoes and coffee. The affordability and accessibility of these agrochemicals are key problems in different parts of the country [16, 71]. In 2019, the national average of pesticides application were below 1kg/ha [71]. In general, high amount of pesticides applied are fungicides 75%, while the remaining 25% contains different insecticides and few herbicides [16]. Mancozeb and Ridomil are the most fungicides applied in agriculture occupying more than 90% of the all applied fungicides [16]. From 2001, the quantity of pesticides applied in the country has been increased at an alarm rate from less than 1000 tons/year to 6000 tones/year in 2019. From 2005, the rate of pesticide application has been increased at very high rate [4, 7]. Dithiocarbamates and pyrethroids pesticides are the most fungicides and insecticides applied respectively (figure 6 and appendix I.*vide infra*).

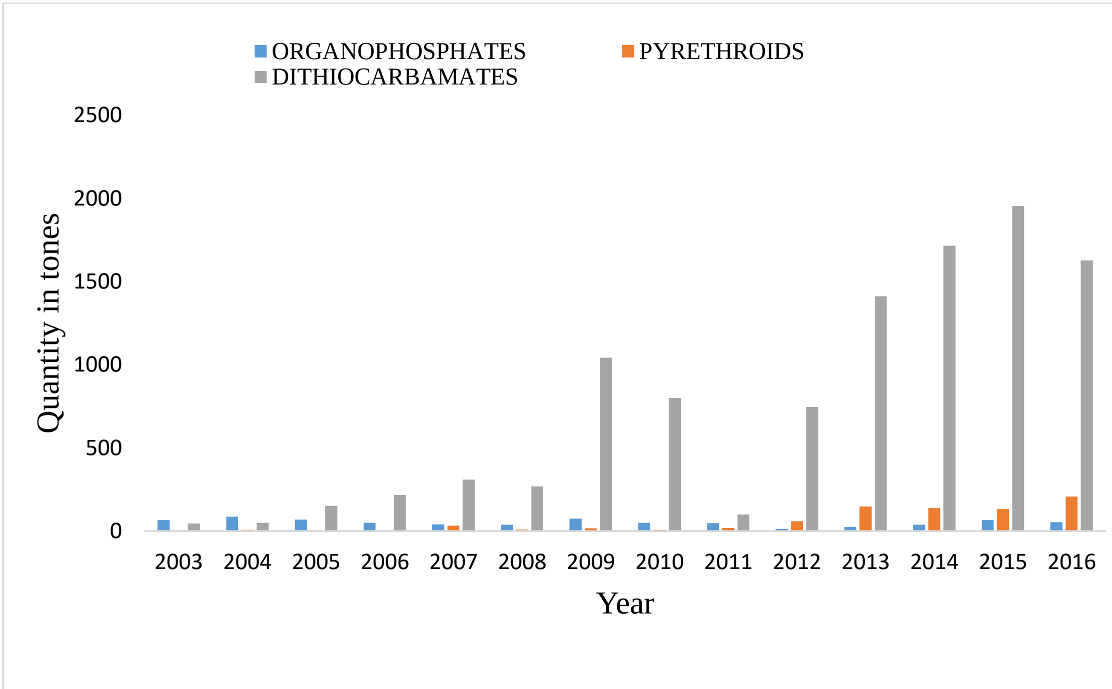


Figure 6: Variation of most applied pesticides in 14 years

Source: FAOSTAT [4]

Chapter 3: MATERIALS AND METHODS

3.1. Data Collection

The data collection from farmers, pesticides sellers and crops and vegetable sellers was conducted through a survey in Eastern province of Rwanda which is located on 1.7819^{o} S, 30.4358^{o} E coordinates (Figure 7). The aim of this survey was to know types of pesticides applied on crops, to assess their application practices in agriculture and to know the way they are handled by all farmers, sellers and crops consumers. Garmin GPS was used to mark the coordinate's location for every participant in the survey. A survey was conducted using a questionnaire (Appendix II.1. *vide infra*) which was developed according to the study objectives. A number of 272 farmers respondents and 21 agro-dealers, chosen randomly, was interviewed to collect information on pesticides management and use.

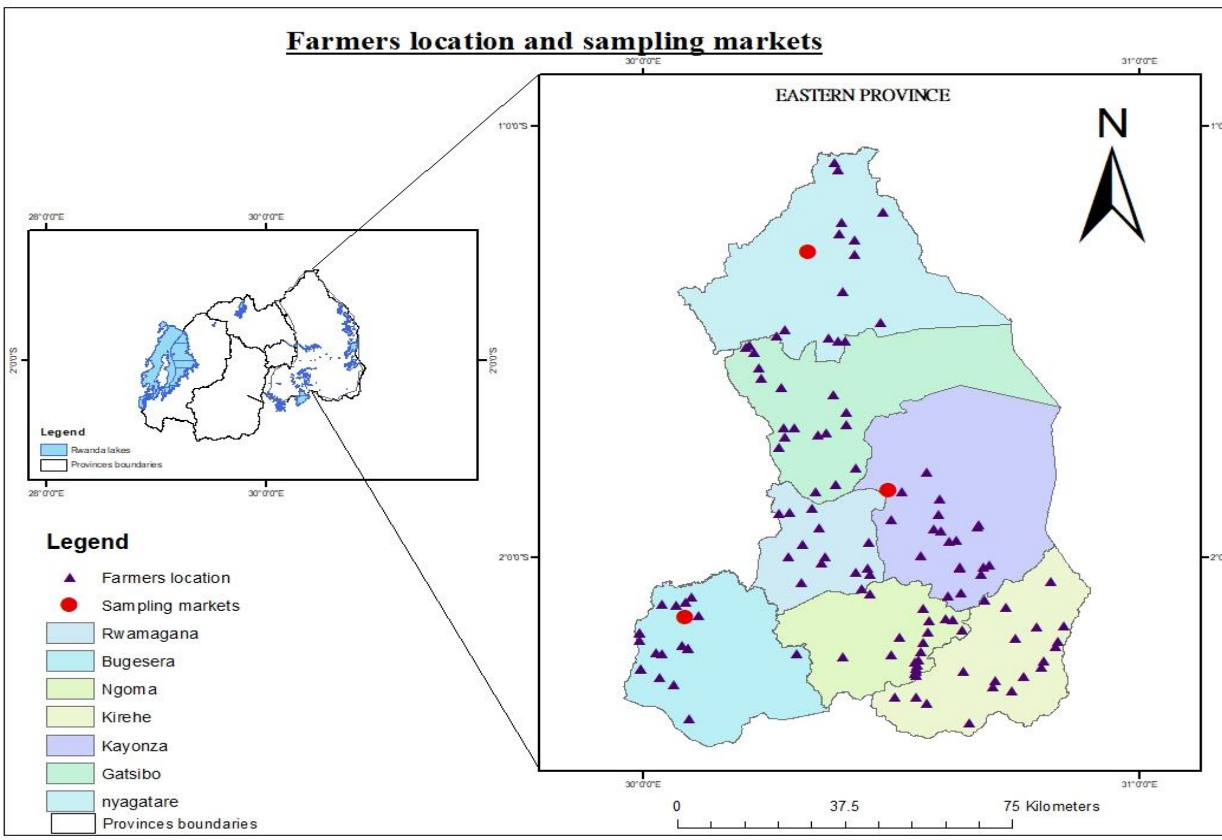


Figure 7: Farmers locations and sampling markets

3.2. Sampling of fruits and Vegetables

The consumption rate of the fruits and vegetables was evaluated by conducting a short interview to consumers before crops sampling. This interview was focused on the quantity and frequency of consumption of fruit or vegetable consumed during two successive days (appendix II.3. *vide infra*). The collected information also included the number and age of household members. Tomatoes, cabbages and oranges samples were randomly collected from three biggest markets in Eastern province of Rwanda which are Nyagatare, Kayonza and Nyamata. Five duplicated samples for every selected crop (tomato, cabbage and orange) were taken by zig-zag method in five points of every selected market in eastern province of Rwanda. Then a total of 45 duplicated samples (15 for every crop) were packed in polyethylene closed bottles or bag, properly labeled and transported in cool box to the laboratory for analysis. The quantity taken for each sample was between 500 -1000 g and in order to avoid sample contamination, each sample was collected in its individual polyethylene bag or bottle.

3.3. Extraction of pesticides residues in crops' samples

Samples were reduced into small pieces by using knife and then grinded with a blender in order to obtain a homogeneous sample. Extraction of pesticide residues from the vegetable and fruit samples was done according to the modified method used by D. Zuccari and I. Vassilieff [75]. A 50 g of each homogenized sample were placed in separate 200 ml conical flask and 2.5 g of NaCl were added to concentrate the extract and then 100 ml of acetonitrile solvent were also added. The sample mixture was shaken for two hours on a shaker with 100 cycles/min before its filtration by using whatman filter paper and washed twice with 20 ml of acetonitrile. After filtration, the filtrate was collected in round bottom flask and concentrated to dryness by using rotary evaporator at $50^{o}C$ and then 5 ml of acetonitrile was added for reconstitution of extracts. The obtained mixture was cleaned up through a column chromatography packed with 5 g of silica gel and kept for further chemical analysis

3.4. Instrumentation

HPLC instrument of Shimadzu technology was used for sample analysis. This instrument was composed by SPD-20A Shimadzu UV/VIS detector, CTO-10AS VP Shimadzu column oven, CBM-20A Communication bus module, DGU-20A5 Degasser, LC-20AD Liquid chromatography pump, SIL-20A Auto sampler and C18, Nucleosol 100-5-C18 250 ×3.2 mm column.

3.5. Chemical analysis of pesticides residues in crops' samples

The standards were prepared by using an intermediate standard solution of 100 mg/l for every analyzed pesticide. The five working standard solutions of 0.01, 0.05, 0.1, 1.0 and 5.0 mg/L were prepared in 50 ml volumetric flask by transferring the appropriate amount from 100 mg/l of intermediate mixed standard solution [75–79]. Standard and samples were analyzed by using Shimadzu HPLC instrument with a UV-Vis detector in verified analytical conditions (Appendix. V *vide infra*).

3.6. Quality control of analysis method

Analysis method of these pesticides was developed through matching the requirements and capabilities of the laboratory with different methods cited in literature. Validation of used method after modification was followed in order to check if the method fit to its purpose. Different validation parameters were used to confirm its validity [80].

3.6.1. *Percentage recovery*

The extraction procedure was validated by conducting the spiking of samples with two different concentrations in tomatoes, cabbages and oranges. The reference sample was spiked at 2 levels (0.5 ppm and 0.1 ppm) of the pesticides targeted and pass through the same procedure as the sample. The percentage recovery was calculated as follow;

$$Recovery = \frac{SSR - USR}{SSR} X100$$

Where, ssr–spiked sample result usr–unspiked sample result.

Equation 1: Percentage recovery calculation

3.6.2 *Limit of Detection (LOD) and Limit of Quantitation (LOQ)*

The LOD and LOQ were determined by using calibration standards. Limit of detection was calculated as per International Chemical Harmonization (ICH) guidelines as shown in equation below [80]

$$LOD = \frac{3.3XSD}{S}$$

Where SD is standard deviation of peak area of calibration curve and S is the slope of the calibration curve.

Equation 2: Limit of detection calculation

Limit of quantification can be calculated as per ICH guidelines using following equation,

$$LOQ = 10\,X\,\frac{SD}{S}$$

where, sd is the standard deviation of the peak areas of the analyte and S is the slope of the corresponding calibration curve [80].

Equation 3: Limit of quantification

3.6.3 *Precision*

Precision were determined by using different levels of pesticide concentrations, prepared from independent stock solutions and analyzed. Inter day and intra-day variation were studied to determine intermediate precision of the proposed modified analytical methods. Different levels of pesticides standards concentrations were prepared, three different times in a day and studied for intraday variation.

This exercise was done in three days for determination of inter day precision [80]. The precision was expressed as percentage of relative standard deviation (% RSD). This relative standard deviation was calculated from three replicates of individual pesticide.

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

sd is the standard deviation and Mean is the mean of peak area.

Equation 4: Relative standard deviation calculation

3.7 Risk Characterization

The risk analysis was performed by comparing estimation daily intake of pesticide residues and their acceptable daily intake. The crop consumption rate was performed by asking 3 consumers per crop in every market to assess the consumption rate of selected analyzed crop in eastern province of Rwanda. During this survey, consumers were asked to describe the meals which were eaten in two previous days [21]. The weight of consumed tomato, cabbage and orange was estimated by comparing the quantity reported and the mean mass of one medium tomato, orange and cabbage. The unit weights of the medium tomato, cabbage and orange of 100, 1200 and 120 g respectively as described by IPCS, was used in risk calculations. The average results of consumption of selected crops in three successive days were used to get the consumption of every household member [21].

The pesticide exposure was estimated by calculation of estimated daily intake of every analyzed pesticide and farmer by using the following formula.

$$\mathrm{EDI} = \frac{\text{Crop consumtion X Pesticide concentration in crops}}{\text{body weight}}$$

Equation 5: Estimation of daily intake

The health risk index from pesticides residues exposure was calculated by using the potential health risk index for non-carcinogenic chemicals used by Akoto and al [81]

$$\mathrm{HRI} = \frac{\mathrm{EDI}}{\mathrm{ADI}}$$

Where hri is stands for health risk index, edi stands for estimated daily intake and adi stands for acceptable daily intake.

Equation 6: Health risk index [21].

According to Akoto et al. (2015) when hri is greater than one, lifetime consumption of crop containing the measured level of pesticide could pose health risks [81].

3.8. Data Analysis

The fields data analysis from survey were analyzed by using descriptive statistics and multivariate analysis like Chi-square (test of independence for categorical data). Laboratory data were analyzed by using statistical mean and standard deviation. The risk characterization was performed by comparing Estimated Daily Intake (EDI) and Acceptable Daily Intake (ADI) after their calculations.

Chapter 4: RESULTS AND DISCUSSIONS

The results from 272 farmers and 21 agro-dealers surveyed in Eastern province Rwanda are presented and discussed in this chapter. Quality control of modified used method like percentage recovery, limit of detection, limit of quantification, precision and robustness were also presented in this chapter. Laboratory analysis results from three crops; tomatoes, cabbages and oranges for four insecticides (Abamectin, Cyhalothrin, Cypermethrin and Profenofos) are presented and discussed and finally results for risk characterization are also presented after the calculations.

4.1. Perceptive from respondents

During the survey, the majority of interviewed farmers were males with 59 % while females were 41% (appendix. III *vide infra*). The age of farmers varied between 18 years and 74 years. The average age of interviewed farmers was 38±12.11 years. Horticulture farmers interviewed use pesticides in different crops like; maize, beans, tomatoes, cabbages, irish potatoes, oranges etc. The results showed that, in Eastern province of Rwanda, fungicides, insecticides, herbicides and acaricides are applied at 63%, 34%, 2% and 1% respectively. Mancozeb (39.68%) and metalaxyl (26.98%) are the most applied fungicide in this region of Rwanda (figure 9). Insecticides applied in this province of Rwanda are dominated by Cypermethrin (48.41%), Profenofos (30.16%) and Abamectin (11.11%) (figure 10).

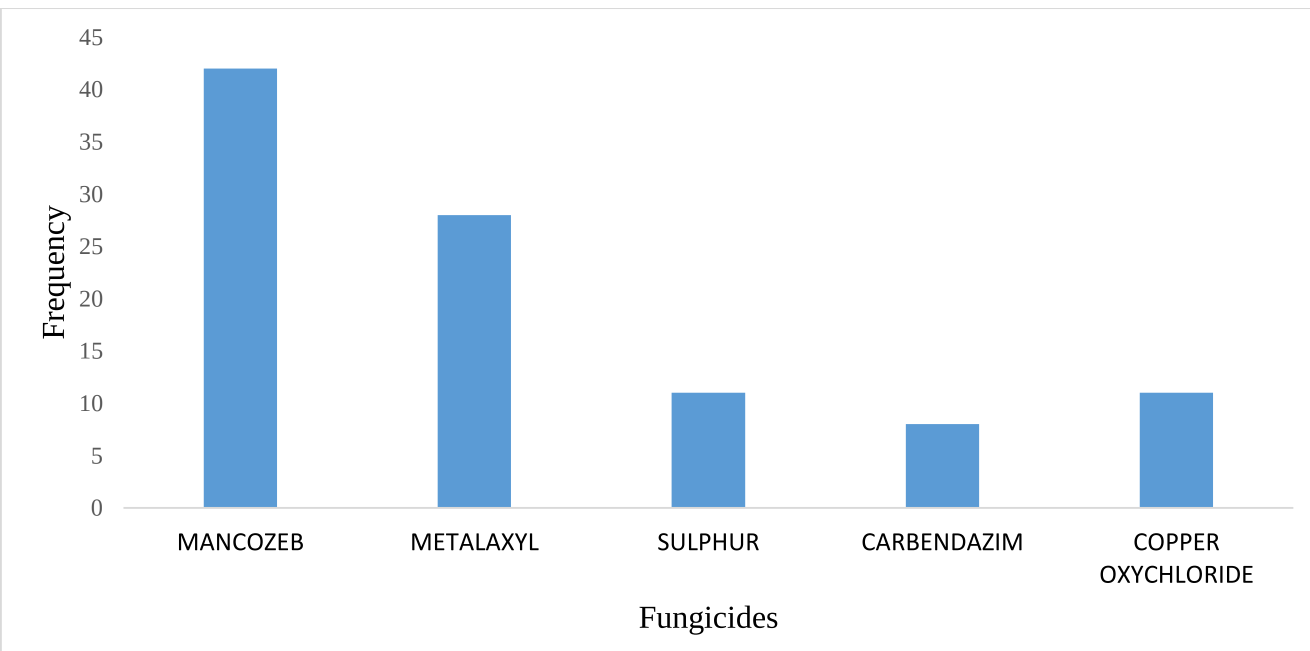


Figure 8: Fungicide used in eastern province of Rwanda

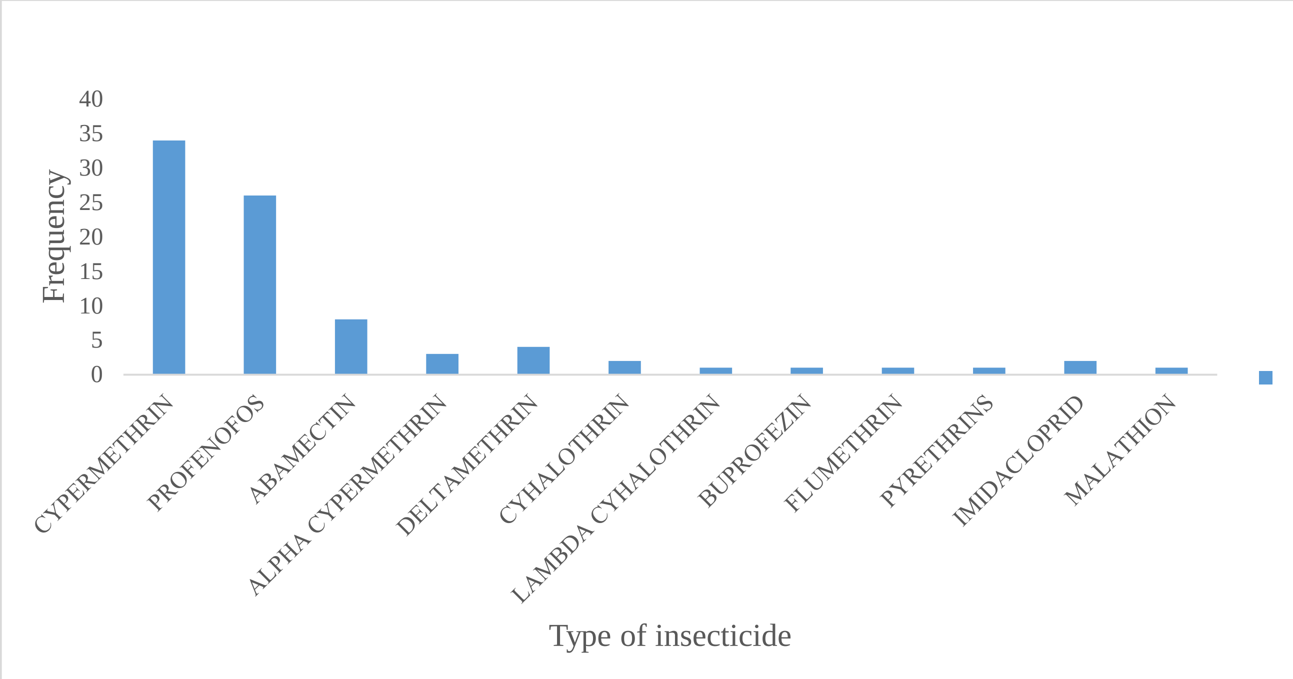


Figure 9: The most insecticides used in Eastern province of Rwanda

Looking on formulation type, these results showed that emulsifiable concentrate (E.C) is the most applied formulation (48%) in Eastern province of Rwanda, soluble concentrate (S.C) and wettable powder pesticide are also used at 35% and 17% respectively.

It was also remarked that in Eastern province of Rwanda, only 31.75% of farmers have training on pesticides using and management of waste from pesticides application. Around 17.46% of farmers in this province are self-trained and by experience they know how to handle these agrochemicals. Many farmers in this area (50.79%) don't know how to manage and handle pesticides products. The observations made on education level of the farmers, in this area, showed that respondents have been mainly attended school at different levels; the primary level (74%), which only 14% and 2% attended secondary school and university respectively. Findings also indicated that 10% of the respondents did not attend school.

Pesticide efficiency on pest control was identified as the main factor leading farmers to use them. Results showed that 83 % of interviewed farmers selected pesticides according to their efficiency on pest control while 15 % explained that their choice was guided by cost and 2 % of respondents, indicated that both the availability and easy using were the leading parameters.

During spraying activities, only 8.73% wear all necessary personal protective equipment, 38.09% are partially protected with one or two personal protective equipment like covering their mouth and nose, wearing protective shoes, hand gloves, covering head, full covered head, goggles, wearing full covered clothes, and 53.18% of Eastern province Rwanda farmers' spray pesticide without any personal protective equipment except normal clothes. As we observed during the survey, when the weather is hot farmers remove their clothes and spray without covering their upper body. Before and after spraying, 58.73 % of farmers kept the agrochemicals in appropriate place, 24.6% of farmers kept them in their living room and 16.67% of farmers kept these agrochemicals with other goods including food.

According to the spraying preparation, 64.28% of the farmers diluted these products as indicated on the containers bottle and 35.72 % diluted differently as indicated on their containers bottles. During spraying activities, 46.04 % of the farmers used hand sprayer as spraying materials and back pack sprayers are used by 53.96% of the farmers.

According to spraying time, 36.51% sprayed in the morning, 34.92 % sprayed at noon while 28.57% considered the evening as the best moment for spraying pesticides. After spraying activities, 76.19% of farmers wash body and clothes, 17.46% wash either body or clothes and 6.35% never wash neither body nor clothes after spraying. In the aim of looking how external persons and animals were directly protected against contamination by sprayed chemicals, 48.41% of respondents confirmed to allow persons and animals entering in immediately sprayed area while 24.6 % sometime respected the restricted entering interval and finally 26.98 % completely respected the restricted entering interval. During spraying preparation, 69.05% of the farmers prepared the spray in the farm, 5.55% did in the storage room and 25.40 % of the farmers conducted this practice in the place close to the water source.

During crops harvesting, only 40.47% of the respondents declared to have respected pre-harvest interval, 22.22% sometime respected pre-harvest interval and finally 37.3% never considered this interval. In this province of Rwanda, 34.92 % of farmers managed pesticides containers in good manner or incinerated these containers, 14.28% reused these containers in domestic activities and 50.79 % dumped the pesticides containers in the environment.

In Eastern province of Rwanda, 56.35% of farmers presented acute toxicity after spraying activities and 43.65% don't present the health effects. During or after spraying activities, health problems reported by many farmers were headache, allergy on exposed part of the body, nausea, dizziness and loss of appetite (Appendix. III *vide infra*).

Only 57.1% of agrochemicals premises complete the requirements of location. 42.9 % of agrochemicals shops are around the residential and commercial areas or mix agrochemicals with food products. Only 23.8 % of workers in agrochemicals shops wear protective clothing. 62% of agrochemical shops don't have first aid facilities equipment. For responsible of these shops, only 81% have the required knowledge on safety management and handling of agrochemicals. In the agrochemicals shops only 28.57% have the required records of agrochemicals supplied to the farmers. In Rwanda only 14.28% of agro-dealers give all necessaries information to the farmers.

These results from the survey are based on responses and field observations of 272 farmers and 21 agro-dealers from eastern province of Rwanda. Mancozeb, Cypermethrin, Profenofos, Abamectin and Cyhalothrin are pesticides most applied in Eastern province of Rwanda similarly to the results found by Nziza, Areco-rwanda [16]. The survey showed a number of banned pesticide in European union but still applied pesticides in the study area like; Mancozeb, Abamectin, Cyhalothrin,cypermethrin, chlorpyrifos, …. These pesticides are classified in highly hazardous and may affect health of farmers and biodiversity [16, 17]. Cypermethrin, Cyhalothrin and Profenofos are highly toxic to bees and this may have negative effects not only to the farmers but also apiculture farming is affected. This effect also goes to the pollination phenomena. Abamectin is highly toxic to bees and make acute toxicity (fatal if inhaled) [17]. Mancozeb and Carbendazim make long term effects [17]. Highly hazardous pesticides are very toxic to beneficial insects like which play the role in pollination and a phase out plan of these compounds is necessary in order to have a safety farming and sustainable agriculture. The results from research realized on Side effects of different pesticides by M. Castillo and C. Avilla (2013), showed that Cyhalothrin is still harmful after 11 weeks of application, Abamectin reduce its harmful between one and six weeks [82]. The negative effects of using these highly hazardous pesticides increase when farmers don't use appropriate spraying materials and personal protective equipment [72].

In this province, there is a slight difference between men and women participating in horticulture agriculture. Males are more occupied by this kind of farming comparatively to the females. Similary to the results found by J. Havukainen et al. (2017) in Vietnam [83]. This kind of agriculture is classified in the income generating crops and due to the history of Rwanda, men are occupied by crops generating money. Another issue is the capacity of women to manage sprayers materials like pump sprayer [84]. Women are mostly occupied by household activities compared to men.

Pesticides management and handling require a good understanding of these products, their effects in the cases of misuse or overuse, the requirements in the storage, transportation, mixing, applying, cleaning used materials and how to manage pesticides poisoning by accident [47].

E.C and W.P are the most applied formulations in Eastern province of Rwanda. Concentrated formulations are very preferable and economical when treating large areas but this form of formulation challenges in performing required dilution for small areas. E.C affects farmers by presenting the dermal hazards, the reason why in management of concentrate formulation personal protective equipment is mandatory in order to protect farmers' health [33, 39]. In Rwanda, agriculture is characterized by the small plots, reason why the ready-to-use formulations are the best choice due to containing small amounts of active ingredients. The education level of Eastern province of Rwanda farmers is another challenge in handling of concentrate agrochemicals due to the lack of required knowledge on chemical dilution, personal protection and waste management [33]. The W.P pose lower dermal hazard but it can present the effects from inhalation to the applicator during preparation activities [39]. Mouth masks are required to the farmers handling wetable powder formulation.

The education level and training in this province are at low rate and these may lead to the presence of many farmers who don't know how to handle pesticides due to the lack of required knowledge in pesticide management. If agrochemicals products are not well managed at farmers' level, the region may suffer of losing beneficial organisms, farmers contaminated, destruction of biodiversity and these agrochemicals may be present in food as residues [30]. The majority of pesticides used in Rwanda are coming from outside and their labels are in foreign languages which may be a problem to the low educated farmers [30]. Many farmers in Eastern province Rwanda spray pesticide without any required personal protective equipment. As protectives clothing are expensive, the small farmers are not able to buy this equipment and this showed as being the main origin of health effects during or after spraying. The statistical data showed a significant positive correlation between acute toxicity of the farmers and their level of personal protective equipment. The same results were found by M. T. Nguyen (2017) and J.S. Okonya (2019) [83, 85].

Eastern province still has the farmers who keep agrochemicals in their living room and mix the agrochemicals with their daily consumed food. This culture increases the risk of being contaminated by agrochemicals and the pesticides vulnerable like pregnant woman and children are more exposed by this practice [83].

The dilution of concentrate pesticides is an issue in this province, many farmers dilute concentrate agrochemicals differently as indicated on their containers bottles. Excessive in pesticide spraying may be the source of high pesticide residues levels in crops and this is very dangerous to crops consumers and farmers. Inappropriate pesticide mixing also may be the origin of some environmental problems like; increasing of pest resistance and losing beneficial microorganisms [30]. Mixing inappropriate pesticide also may lead to the pest resistance which will be costly to manage [83].

The presence of a big number of farmers spray agrochemicals by using hand sprayer as spraying material is very dangerous. This may be influenced by lack of capital for buying pump sprayer and when you compare the cost of pump sprayer and the value of most crops in the plot area, the pump is very expensive. Most of the time farmers use brush or plant leaves during spraying without gloves or face mask. Hand sprayer method for farmers without pesticide protective equipment may influence pesticide contamination [83].

Farmers' hygiene is also a big challenge, due the poverty of many farmers and they stay in the same clothes and don't wash their body or clothes after spraying. Staying with agrochemicals on clothes or on the body may also increase the risk of being contaminated by agrochemicals [86].

In eastern province, there are many lakes, rivers and swamps containing many living organisms, preparing spray around these water bodies may be the source of environmental pollutions of these water bodies. Agrochemicals are very toxic to aquatic living organisms. This practice must be avoided because during spraying preparation and washing the spraying equipment around water bodies, water may be polluted by chemicals [39].

The climate of spraying time also is crucial in pesticides management. Windy and sunny weather may increase pesticides volatilization and drift which are the major source of pesticide exposures [83]. Most of the farmers in the study area don't consider the wind direction before spraying and also they spray at sunny time. This kind of climate also influences the farmers to remove the protective equipment.

Respecting pre-harvest interval is required in order to minimize the risk of consuming residues in crops. Most of the time, harvest time is determined by market demand, when fruits or vegetable are more requested these crops may be harvested even directly after spraying. Harvesting crops without respecting the required time for pesticide degradation is very dangerous to the farmers and consumers. Consumers are highly exposed to residues if farmers doesn't observe pre-harvest interval [51, 87].

Waste management in studied area is also a critical point. Many farmers dump waste generated in the environment or reuse in the domestic purposes. The dealers in Rwanda sell agrochemicals in the small container of pesticides and this system is good to the farmers with small land area like Rwandan farmers who can purchase the quantity appropriate to the farm size. However, the management of waste generated is a big challenge [30]. In this province, we don't have a good strong waste management system. Dumping agrochemicals in the environment is very dangerous to the biodiversity. The lack of appropriate waste management system which leads the farmers to dispose the empty containers in the farms or preparation area could put the farmers and all biodiversity at high risk. This practice is the main problems of pesticide application and management in developing countries [72].

The results on Health effects of the farmers, showed a big number presenting side effects after spraying. The statistical data showed a significant positive correlation between acute toxicity of the farmers and their level of personal protective equipment, training and education with p-value of 0.001, 0.006 and 0.003 respectively (appendix III.3. *vide infra*). The same results were found by M. T. Nguyen (2017) and J.S. Okonya (2019) [83, 85]. Results of health effect from pesticide application are similarly to the finding of 90% by B. Ndayambaje (2019) [88] in rice farmers from Nyagatare district in eastern province of Rwanda [15]. The most reported symptoms associated to pesticides contaminations are the common manifestation of acetylcholine enzyme inhibition. The reported health effects are justified by the type of pesticides used and the formulation type. Dermal and inhalation contaminations are the most reported contaminations [86]. As reported by P. Hutter et al., (2021) and D. Kumari et al.,(2021), pesticides farm workers are more exposed to the pesticides in their daily activities and this may lead to the acute toxicity of the farmers [88, 89]. In the study area the safety measures have to be improved in order to protect farm daily workers.

4.2. Detection of pesticides residues from tomatoes, cabbages and oranges of the Eastern Province of Rwanda

4.2.1 *Quality of the Method Used*

The percentage recovery was between 59.8 to 76.1%. Residues in orange showed a high percentage recovery compared to tomato and cabbage. Residues in cabbage showed less percentage recovery. The 42% showed a percentage recovery more than 70% and 58% showed less than 70%. Cypermethrin and Cyhalothrin showed the best recovery. Profenofos showed less recovery compared to other pesticides (Appendix. VI *vide infra*). With the optimized conditions, the analyzed pesticides (Cypermethrin, Cyhalothrin, Profenofos and Abamectin) showed a good linearity between 0.01 ppm and 5 ppm with the correlation between 0.9991 and 0.9998. The detection limit ranged between 0.002 to 0.01 and the limit of quantification ranged between 0.01 to 0.03ppm (Appendix. XIII *vide infra*). The results of RSD showed that; Cypermethrin has a good repeatability both intra-day and inter-day of 1.8 and 3.18 respectively. In four analyzed pesticides, Abamectin was the least precise with 8.2 and 15.4 both intra-day and inter-day respectively (Appendix. XIII *vide infra*).

From these obtained results it was remarked that the used modified method was rapid and accurate to determine Cypermethrin, Abamectin, Cyhalothrin and Profenofos in tomatoes, cabbages and oranges. Acetonitrile, as used solvent, has shown good extraction efficiency for all selected pesticides compared to other assessed solvents including dichloromethane, cyclohexane and acetone. The same results were found by W. Xie et al.(2006) and O. Akoto (2015) [81, 90, 91]. As the results of RSD were below 20%, the method is very precise [92].

The calibration curves of all analyzed pesticides showed a correlation coefficient more than 0.999 confirm the linearity of the method. The detection and quantification limits of all analyzed pesticides were below their maximum residue limits and this confirmed the using of the method to determine the pesticides residues. The analyzed pesticides had similarities in their chemical structures and this explains the possibilities of using the same procedures in extracting, clean up, and elution in HPLC machine [92] . The percentage with a percentage recovery less than 70% showed good precision with consistent results and this confirmed accuracy of this method in residues determinations [92]. The silica gel used to clean-up extracted pesticide residues indicated its efficiency in removing interfering substances and led to pure extracts.

4. 2.2. *Presence of the pesticides residues in the analyzed crops*

Ninety samples of tomatoes, cabbages and oranges were collected from 3 markets of the Eastern province of Rwanda (Nyamata, Kayonza and Nyagatare) to determine the residues of Cypermethrin, Abamectin, Cyhalothrin and Profenofos. The results of pesticide residues found are presented (in appendixes. IX *vide infra*) for tomatoes, cabbages and oranges, respectively. To start analysis, all used standard were injected in HPLC and their peaks recorded as references during further analysis of the extracts (Figures 11-13).

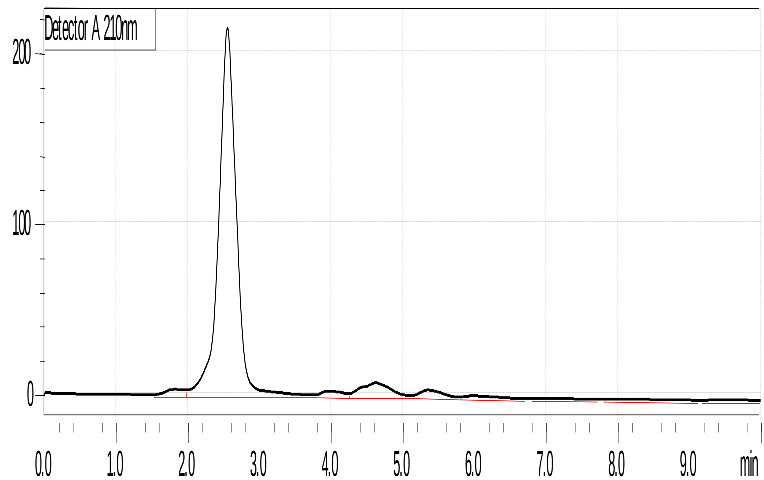


Figure 10: Standard peak of Cyhalothrin

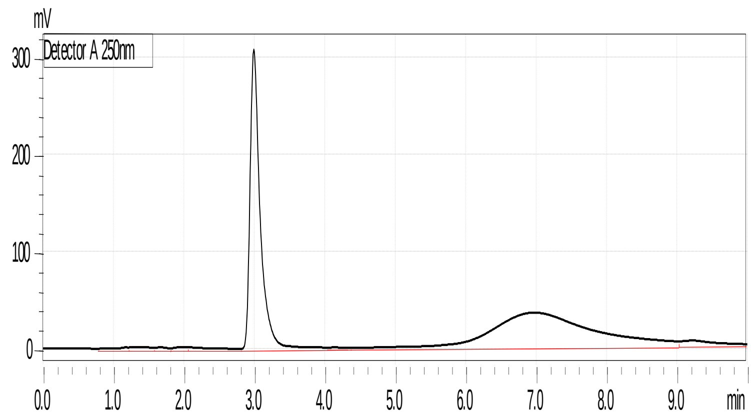


Figure 11: Standard peak of Abamectin

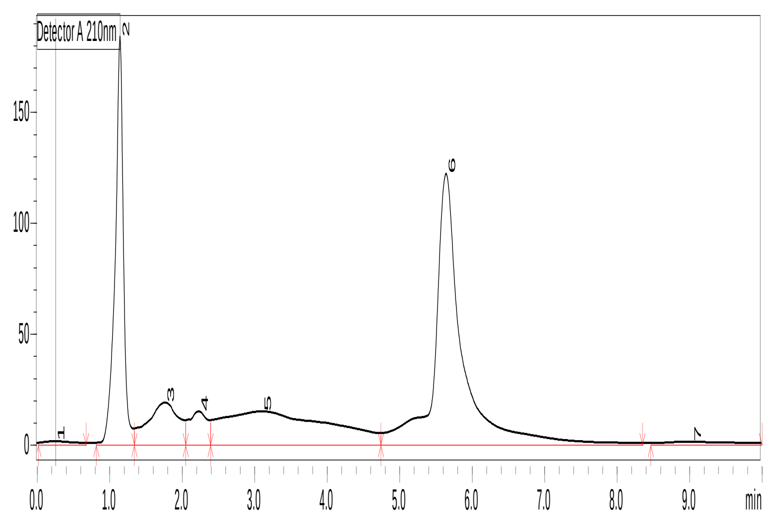


Figure 12: Standard peaks of Cypermethrin(2) and Profenofos(6)

Pesticides residues in tomatoes

In tomatoes, out of 30 samples analyzed only 7 (23%) samples presented pesticides residues and 23 samples (77%) of tomatoes contained no detectable values of pesticide residues. Three pesticides (Cypermethrin, Abamectin and Profenofos) are detected in tomatoes samples. The most detected was Cypermethrin in five samples (16.6%) and Cyhalothrin was not detected in all tomatoes samples. Two pesticides (Cypermethrin and Profenofos) were detected at the same time in two samples. The high value of residue in tomatoes was detected for Abamectin (1.251 mg/kg) from samples taken at Nyagatare market. Nyagatare market indicated the presence of three pesticides; Cypermethrin, Abamectin and Profenofos in tomatoes while only one pesticide, Cypermethrin, was detected in the samples taken from Nyamata market with 0.599 ppm.

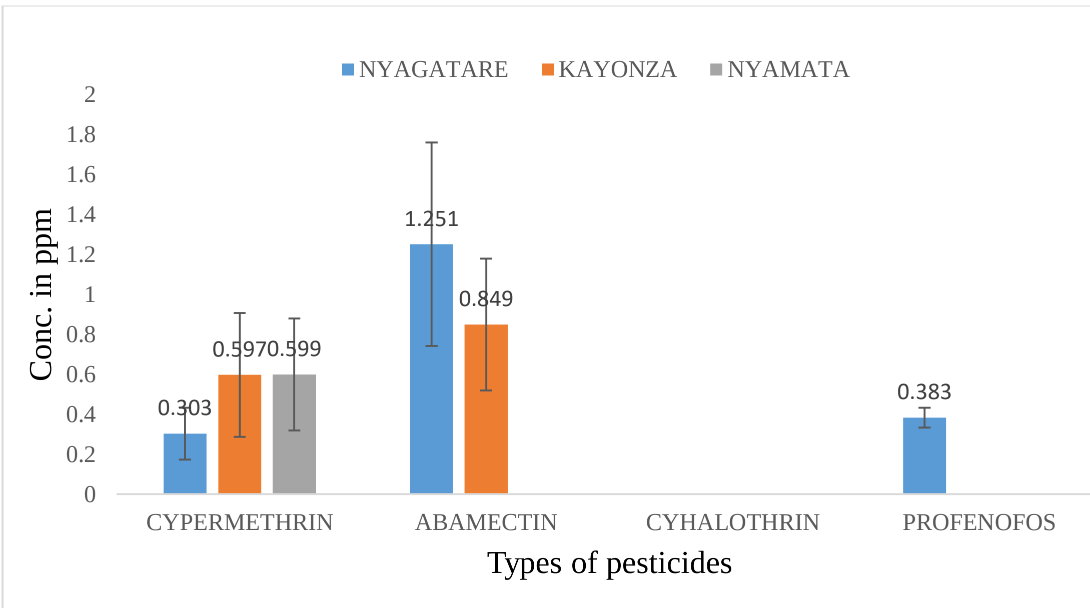


Figure 13: Mean of pesticides residues in tomatoes samples from 3 markets

Pesticides residues in cabbages

In 30 samples of cabbages analyzed, only 4 samples (13.3%) presented the pesticide residues. All analyzed pesticides are detected in cabbages. Cypermethrin and Profenofos are detected at the same time in two samples. Cyhalothrin and Abamectin are detected in one sample. The high values of residues in cabbage were detected in Nyagatare market are 0.665ppm and 1.064ppm of Cypermethrin and Profenofos respectively. The less detected residue of Abamectin was detected in Kayonza market.

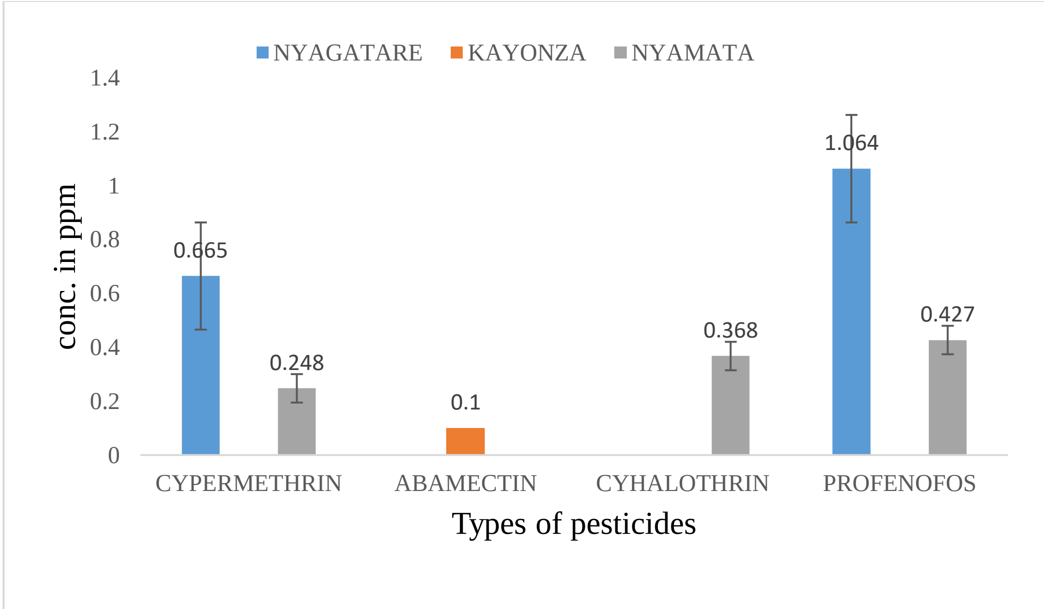


Figure 14: Mean of pesticide residues in cabbages samples from 3 markets

Pesticides residues in oranges

Four out of 30 samples of oranges presented residues. In orange samples, no one presented two pesticides residues at the same time. Three pesticides (Cypermethrin, Profenofos and Abamectin) are detected in orange samples. Cyhalothrin was not detected in all orange samples and Abamectin was detected in two orange samples. The high value of pesticide residues of Profenofos 1.193ppm was detected in Nyamata market and the least detected pesticide residues of Cypermethrin was detected at Nyagatare market.

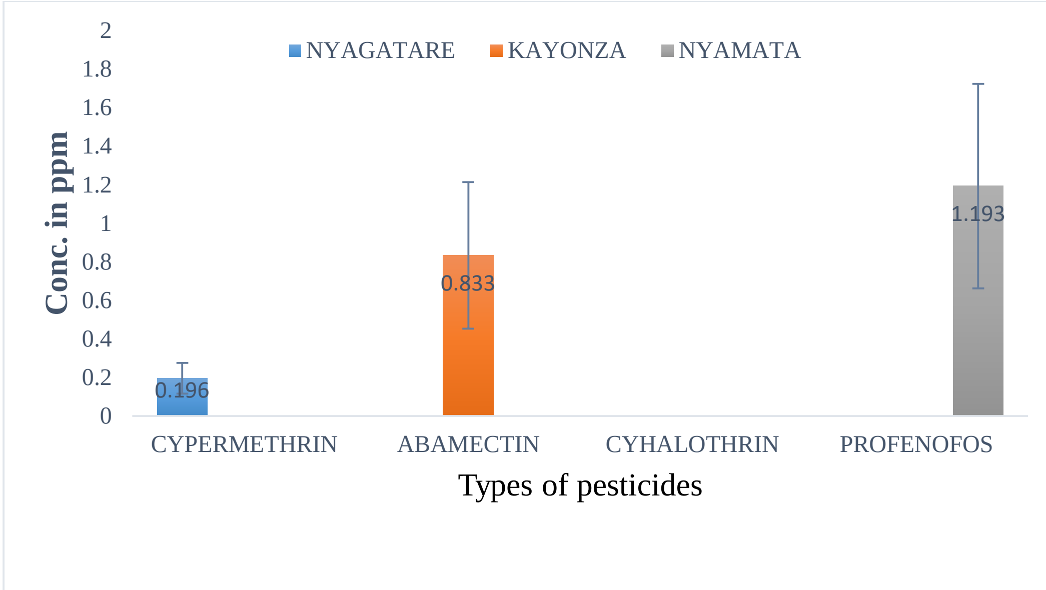


Figure 15: Mean of pesticides residues in orange samples

From the above results on the presence of pesticides residues in cabbages, tomatoes and oranges, Cypermethrin which is an active ingredient of most insecticides used in Rwanda, was the most detected and the same results were found by M. Jankowska (2011) [93]. This can be confirmed by the results from the conducted survey in this study about the high quantity of this pesticide applied in agriculture farming in Eastern Province. The same results have been reported by Areco-Rwanda nziza,2020 [16] about its dominance use in the whole Country. The pesticides residues found in tomatoes were a little different of the founding of M. Kimanya et al (2016) [21].The results from orange are a little below to the results found in research of V. Nakano (2016) [94]. Multiple pesticides residues detected in tomatoes (Cypermethrin and Profenofos) are explained by the combination of Cypermethrin and Profenofos as active ingredient in most insecticides used in Rwanda. These results also are explained by the frequency of pesticides application in tomatoes. The results of residues confirmed that the applied pesticides remain present on the surface of tomatoes for a certain time.

4.3. Characterization of the contamination risk of end users of pesticides

Characterization of the contamination risk of the end users of pesticides was done using data from the survey realized on consumers. During samples collection, a sample of respondents who were found to buy vegetables and fruits was taken and responded to the consumed quantity of these vegetables and fruits per day. The probable quantity of pesticides residues consumed by adult household member from the study area was calculated by using consumption rate and 70 kg as estimated adult weight[81]

(Appendix. X *vide infra*). The high mean concentration was used for estimation of the daily intake concentration calculation.

Obtained results indicated that per capita consumption of these selected crops was between 19 to 65 g/day (average, 35.88±14.39) for tomatoes, 0 to 60 g/day (average, 16.88± 22.67) for cabbage and 0 to 30 g/day (average, 6.55± 10.80) for orange (Appendix.IV.*vide infra*). These findings on calculated health risk showed no risk of consuming pesticides treated crops found in Eastern province of Rwanda as the ratio of Estimated Daily Intake to Acceptable Daily Intake was less than 1. The health risk index calculated for all analyzed pesticides were less than one and this indicated no issue in lifetime consumption of tomato, cabbage and orange sold in Eastern province of Rwanda [81, 95]. However considering the hydrophobicity of these chemicals [96, 97] , many investigations are needed to assess their possible storage and accumulation in consumers tissues and the risk of causing illness after a long time of consummation.

Chapter 5: CONCLUSION AND RECOMMENDATION

5.1. Conclusion

The work focused on assessing the use practices of pesticides in Eastern province of Rwanda, the application methods by farmers, possible negative effects on the end users, and possible presence of Cypermethrin, Cyhalothrin, Abamectin and Profenofos residues in ones of the highly consumed crops in that region. The results showed that Mancozeb and Cypermethrin are the most applied pesticides in the study area with lack of required personal protecting equipment for farmers and some of dealers, leading to their possible intoxication. The management of the generated wastes during spraying of these chemicals, and the way mixing is done were also found as the major sources of environmental pollution. The analysis of Cypermethrin, Cyhalothrin, Abamectin and Profenofos by using HPLC instrument, showed a good precision, linearity, limit of detection and limit of quantification. All four analyzed pesticides were detected in three selected markets but with no serious risks on health of the consumers. However, as these chemicals are mainly lipophilic and therefore like to be stored in fat when ingested, other further investigations on biological samples are needed.

5.2. Recommendations

In Rwanda, more than 70% of population relies on farming, increasing of farmers' awareness on pesticide application, handling and safe management is a good option on pesticide application. Protective equipment and sprayer materials are more expensive to the small farmers; government can provide a "nkunganire" on these materials. The government of Rwanda should plan the way of phase out of these highly hazardous pesticides which are most used in Rwanda. In order to reduce the impact of highly hazardous pesticides, implementation of integrated pest management is needed.

We recommend also a joint collaboration between farmers and researchers in order to improve farmers' knowledge on appropriate pesticide and develop integrated pest management. Further research on assessing pesticide residues in many crops is required in order to know their level in food residues. The determination of maximum residue levels of applied pesticides also is required. Pesticides pollution control on surface water and soil are also required. The government of Rwanda should implement a strong system of risk assessment in order to show the risk levels and the strategies of management the risk.

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APPENDIXES

APPENDIX I

Pesticides applied in Rwanda in 30 years in tones (source FAOSTAT)

YEAR	Total	Insecti cides	Herbi cides	Fungi cides	Organopho sphates	Pyreth roids	Dithiocarba Mates
1990	157	19	0	138			138
1991	107	11	0	96	10		96
1992	97	25	13	59	25		57
1993	127	38	11	79			
1994	158	51	9	98			
1995	188	64	6	118			
1996	219	77	4	137			
1997	249	90	2	157	88	1	154
1998	157	103	0	54	98	5	37
1999	152	70	2	81			
2000	147	36	4	107	30	5	96
2001	72	69	1	1			
2002	106	71	2	32			
2003	141	74	3	64	68	4	46
2004	160	94	6	60	86	7	49
2005	223	70	0	153	69	1	152
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

Continued

2016	2027	317	1	1709	53	207	1627
2017	2027	317	1	1709			
2018	2027	317	1	1709			
2019	2027	317	1	1709			

APPENDIX. II.

II.1 Questionnaire used during farmers survey

District:……………………Sector………………..GPS:.……………/………

Farmer'sname:……………………………………………age………sex…….

CROP …………………………….

1. Which pesticides do you use?………………..……………….................... ………
2. Why do you select these pesticides?

A. cost B. efficacity C. availability D. easy to use E. no reason

3. How do you store your pesticides?

A) appropriate store B) In the living room house C) with the food

4. Education level

A. Never attend school B. primary school C. secondary school D. university

5. Have you received any training on pesticide management?

a) Yes b) self-trained c) no training

6. Frequency of spraying per crop

a.< 2 b. 2-4 c. 5-6 d. > 7

7. What do you wear while applying pesticides?

A. shoes B. mask C. head cover D. full sleeve shirt E. glasses F. gloves G) nothing

8. Do you take a bath right after spraying? A) yes B) sometimes C) no
9. Do you changes clothes right after spraying? A) yes B) sometimes C) no
10. Person or animal is allowed into freshly sprayed areas?

A) yes B) sometimes C) no

11. Food product is consumed immediately after application of pesticides?

A) yes B) sometimes C) no

12. Do you respect pre-harvest intervals before crop harvesting?

A) yes B) sometimes C) no

13. Spraying time A) morning B) Noon C) afternoon D) all the time
14. type of applicator A. hand sprayer B. backpack sprayer C. tractor D. other
15. How do you dilute your pesticides? Is it the same as the indication on label?

A. it's the same B. not the same

16. Do you check the weather forecast?

A) Yes, I never spray when it is raining, windy or too hot

B) Yes, but often I need to spray anyway C) No

17. Do you follow wind direction A) yes B) No

18. Empty pesticides containers are;

A) re-used for domestic purposes B) incinerated C) returned to the dealers

D) dumped in the environment.

19. How do you manage the expired pesticides?

A) re-used B) stored in good conditions for incineration C) dumped in the environment.

20. Where do you prepare the spraying solution?

A. In the field/on the farm; B. In storage room; C. Living area; D. Close to the water source

21. How close to a water pond do you spray pesticides?

A. 1meter B. 5 meters C. 10 meters D. 20 meters E. more than 20 meters

22. Possible health effects after spraying

A. headache B. sneezing C. vomiting D. stomach ache E. skin rash F. back ache G. dizziness H. diarrhea I. Nausea J. blurred vision K. eye irritation

II.2 Questionnaire used during a Agro-dealers survey

Province:…………… District:……………………Sector………………..

GPS:X….……………Y……………………

AGRO-DEALER'Sname:……………………………………………

1. location of agro-chemicals

  a. Away from residential areas, hospitals, shopping areas and food manufacturers.
  Yes…………………no…………

  b. Away from watercourse, open storm, water channels or water catchment.
  Yes………………..no……………………..

  c. Away from areas which are subject to flooding.
  Yes……………no………………

2. Workers and operators in premises are protected

  a. Wear protective clothing yes……….no……….

  b. Premises are well equipped with appropriate first aid facilities. Yes……..no…..

3. Responsible has required technical knowledge on

  a. Chemistry b. toxicology c. safety and general use of agrochemicals d. none

4. Records on agrochemicals sold are kept in good manner

  a. Everyday b. sometimes c. No records

5. Responsible gives all necessaries information to the farmers

a) Every time b) sometime c) none

II.3 Questionnaire used in consumer's survey

District:……………………Market

0. How many personal in your household?........
1. Sex associated to the age ………..age…………….
2. Food consumed in 24 hours ago 1. Tomato……….. cabbage………. orange…….
3. Food consumed in 48 hours ago 2. Tomato………….cabbage………orange……..
4. Food consumed in 72 hours ago 3. Tomato …………cabbage ……….orange……..

APPENDIX. III

III.1 Results from farmers' survey

			Frequency (N=272)	Percentage (%)
1. Pesticides applied	Insecticide(34%)	Cypermethrin	132	48.41
		Profenofos	82	30.16
		Abamectin	30	11.11
		Cyhalothrin	15	5.55
		Other	13	4.76
	Fungicide (63%)	Mancozeb	108	39.68
		Metalaxyl	73	26.98
		Copper	30	11.11
		Sulphur	24	8.73
		Other	37	13.49
	Herbicide (2%)			
	Acaricide (1%)			
2. Crops surveyed	Tomato		37	13.8
	Cabbage		35	12.9
	Orange		17	6.1
	Beans		33	12.0
	Maize		37	13.8
	Potatoes		30	11.2
	Aubergine		26	9.5
	Others		56	20.7
3. Pesticides storage	In appropriate store		160	58.73
	In the living house		67	24.60
	with the food		45	16.67
4. Farmers education	Never attend school		28	10.32
	primary school		201	73.81
	secondary school		37	13.49
	University		6	2.38

Continued

5. Farmers training	self-training	48	17.46
	Received training	86	31.75
	no training	138	50.79
6. Spray Preparation Area	In the field/on the farm	188	69.05
	close to the watersource	69	25.40
	in storage room	15	5.55
7. Frequency of spraying	< 3 times	82	30.16
	3-6 times	110	40.47
	>7 times	80	29.36
8. Protection equipment	Full protected	24	8.73
	partial protected	104	38.09
	no protection	144	53.17
9. Farmers hygiene	Wash body and clothes	207	76.19
	Wash body or clothes	48	17.46
	Never wash body nor clothes	17	6.35
10. Respect PHI	always respect	110	40.47
	sometime respect	60	22.22
	never respect	102	37.3
11. Pesticide formulation	WP	47	17.46
	SC	95	34.92
	EC	130	47.62
12. Respect REI	always respect	73	26.98
	sometime respect	67	24.60
	never respect	132	48.41
13. Spraying time	Morning	99	36.51
	Noon	95	34.92
	Evening	78	28.57
14. Spraying materials	hand sprayer	125	46.03
	backpack sprayer	147	53.96

Continued

15. Dilution	The same as the label	175	64.28
	Not the same	97	35.72
16. Wind direction	Observe wind direction	119	43.65
	Doesn't observe wind direction	153	56.35
17. Managing waste	re-used for domestic purposes	39	14.28
	incinerated	95	34.92
	dumped in the environment	138	50.79
18. Farmer's ages	<25	41	15.08
	26 – 40	112	41.27
	41 – 60	76	27.77
	>61	43	15.87
19. Sex	Male	168	61.7
	Female	104	38.3
20. Health effects	Presence of health effects	153	56.35
	absence of health effects	119	43.65

III.2 Results from Agro-dealers' survey

		Frequency (N=21)	**Percentage (%)**
1. LOCATION	A way from residential area	12	57.1%
	Around the residential area	9	42.9%
2. WORKERS PROTECTION	No protective clothing	16	76.19
	Wear protective clothing	5	23.81
3. FIRST AID PROTECTION	Well equipped with first aid	8	38%
	No first aid facilities	13	62%
4. INFORMATION FROM AGRODEALER	Responsible give all necessary information to the farmers	3	14.28%

	Sometimes give information to the farmers	8	38%
	No information	10	47.6%
5. AGRODEALERS' KNOWLEDGE ON CHEMICAL SAFETY	Know safety	17	81%
	Do not know safety	4	19%
6. RECORDS	All records	6	28.57%
	Some records	5	23.8%
	No records	10	47.6%

III.3 Multivariate analysis of health effects and different pesticides exposure

		Total farmers	Farmers Affected	Farmers non affected	Chi-square χ^2	P-Value
Protection	full protected	24	9	15	13.85	0.001
	partial protected	104	58	46		
	non protected	144	86	58		
Training	Trained	48	18	30	10.11	0.006
	Self-trained	86	47	39		
	Not- trained	138	88	50		
Frequency of spraying	< 3 /crop	82	50	32	1.29	0.522
	3 – 6 /crop	110	58	52		
	>7/crop	80	45	35		
Hygiene	Wash clothes and body	207	110	93	6.42	0.040
	Wash clothes or body	48	35	13		
	Neither clothes nor body	17	8	9		
Pesticide formulation handled	W.P	47	28	19	0.31	0.85
	S.C	95	52	43		
	E.C	130	74	56		

Education	Not attend school	28	19	9	13.67	0.003
	Primary school	201	119	82		
	Secondary school	37	15	12		
	University	6	0	6		
Spraying time	Morning	99	52	47	4.49	0.105
	Evening	95	50	45		
	Noon	78	52	26		
Spraying materials	Hand operators	125	77	48	2.69	0.1
	Pump operators	147	76	71		
Wind direction	Observe wind direction	119	60	59	2.92	0.087
	Doesn't observe wind direction	153	93	60		
Sex	Male	168	101	67	0.141	0.706
	Female	104	62	42		
Ages of farmers	< 25	41	21	20	8.128	0.043
	26-40	112	65	47		
	41-60	76	43	33		
	>60	43	24	19		

APPENDIX IV.

Mean of crops consumption results from 3 successive days

	Tomato g/day	Cabbage g/day	Orange g/day
Nyamata	28	18	30
	42	0	0
	25	40	0
Kayonza	19	0	15
	48	60	0
	25	0	0
Nyagatare	65	0	0
	40	34	14
	31	0	0
Mean± Std	**35.88±14.39**	**16.88±22.67**	**6.55±10.80**

APPENDIX. V.

HPLC Analysis conditions

HPLC Condition	Abamectin	Profenofos	Cypermethrin	Cyhalothrin
Mobile phase	60% ACN	60% ACN	60% ACN	60% CAN
Oven temperature	30 ºC	30 ºC	30 ºC	30 ºC
Wavelength	254nm	210nm	210nm	210nm
Flow rate	0.8 ml/min	0.8 ml/min	0.8 ml/min	0.8 ml/min
Injection volume	20 µl	20 µl	20 µl	20 µl
Analysis time	10min	10min	10min	10min
Column used	C18, Nucleosol 100-5-C18 250 ×3.2 mm	C18, Nucleosol 100-5-C18 250 ×3.2 mm	C18, Nucleosol 100-5-C18 250 ×3.2 mm	C18, Nucleosol 100-5-C18 250 ×3.2 mm

APPENDIX. VI.

Pesticides percentage recovery

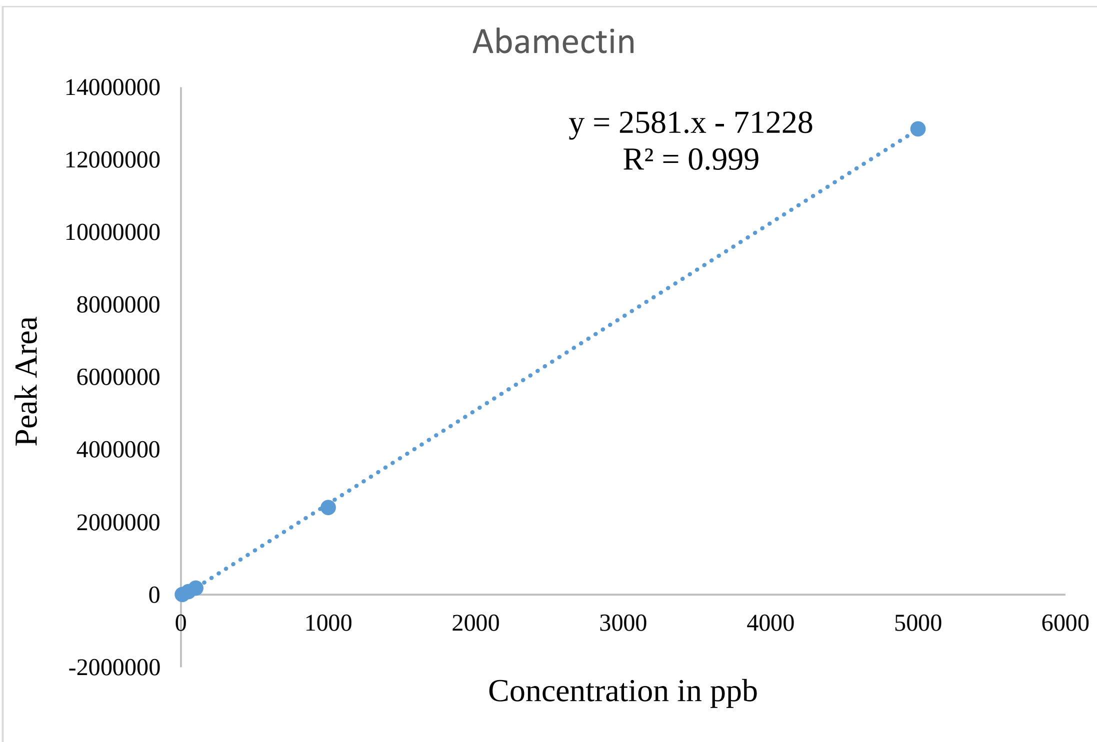
	Abamectin	Cypermethrin	Profenofos	Cyhalothrin
Tomatoes	68.7±0.98	70.4±0.91	63.4±1.6	73.8±2.45
Cabbages	61.8±2.26	65.5±1.76	59.8±1.13	68.1±1.83
Oranges	71.9±1.2	76.1±1.2	69.05±2.75	75.4±0.46

APPENDIX. VII.

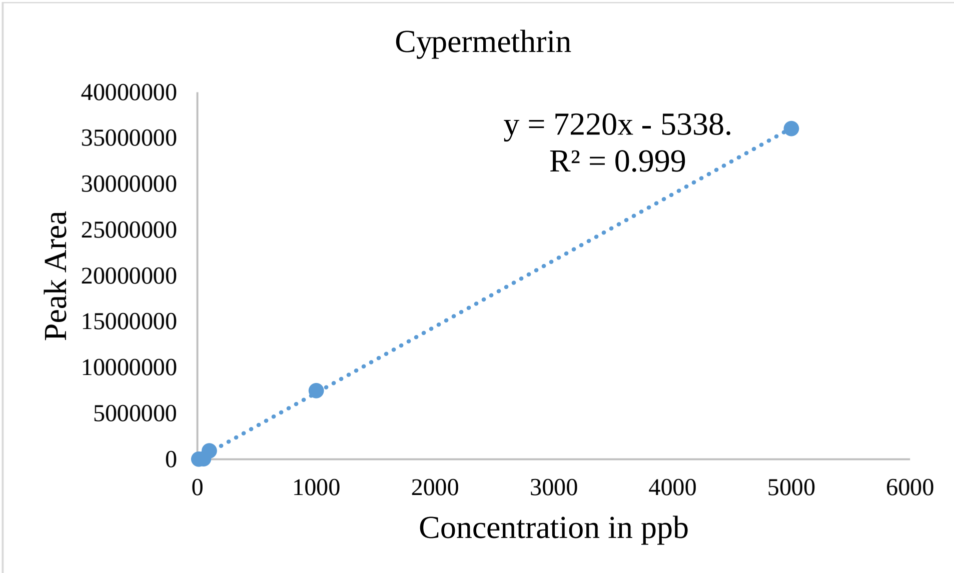
Calibration curve peak area of analyzed pesticides

Standards concentrations in ppb	Abamectin	Cypermethrin	Cyhalothrin	Profenofos
10	7381	8754	6002	4573
50	88653	43672	26446	13644
100	181817	886523	551046	193211
1000	2412841	7465231	4988945	5633014
5000	12856734	36044712	23875124	25441325

VII.1Calibration curve of Abamectin



VII.2 Calibration curve of Cypermethrin



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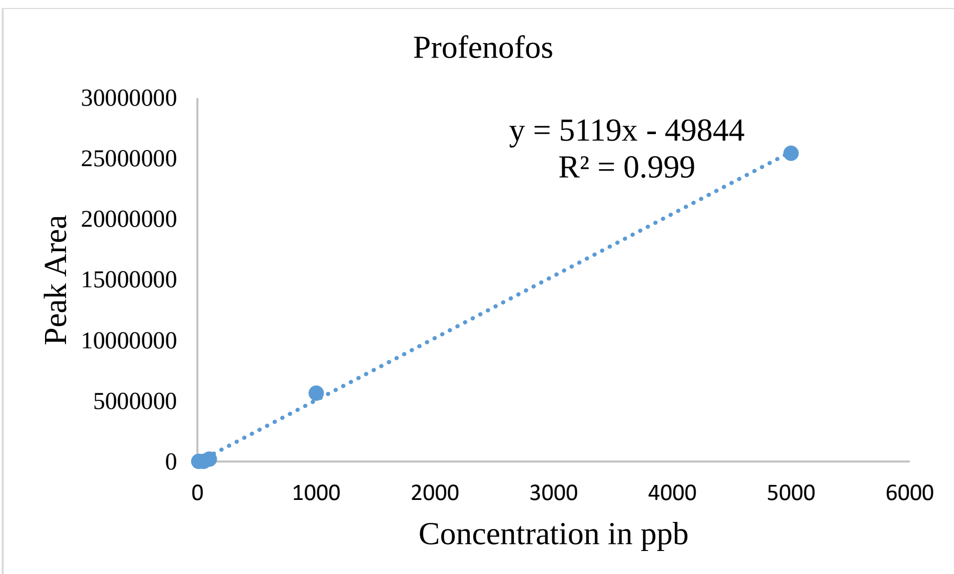
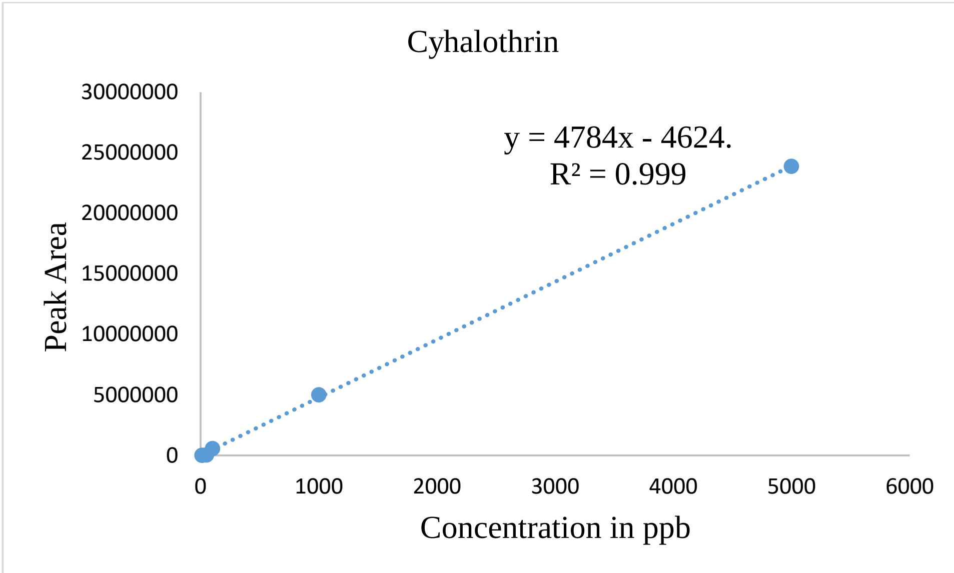
VII.3 Calibration curve of Profenofos



VII.4 Calibration curve of Cyhalothrin



APPENDIX. VIII.

Quality control results

Analyte	Range ppm	Linear equation	Correlation coefficient (r^2)	LOD (mg/L)	LOQ (mg/ L)	Repeatability (RSD %)	
						Intra-day	Inter-day
Cypermethrin	0.01- 5	y=7220X -5338.8	0.9998	0.002	0.01	1.8	3.18
Abamectin	0.01- 5	y=2377.2X-19352	0.9997	0.01	0.03	8.2	15.4
Profenofos	0.01- 5	y=5119.3X-49844	0.9991	0.010	0.03	4.6	5.9
Cyhalothrin	4.01- 5	y=4784.2X-4624.6	0.9998	0.003	0.01	6.6	9.6

APPENDIX. IX.

IX.1 Pesticide residues in tomato

Tomato samples	**Cypermethrin ppm**	**Abamectin ppm**	**Cyhalothrin ppm**	**Profenofos ppm**
NY-S1	BDL	BDL	BDL	BDL
NY-S1	BDL	BDL	BDL	BDL
NY-S2	BDL	BDL	BDL	BDL
NY-S2	BDL	BDL	BDL	BDL
NY-S3	0.209	BDL	BDL	0.379
NY-S3	0.397	BDL	BDL	0.387
NY-S4	BDL	BDL	BDL	BDL
NY-S4	BDL	BDL	BDL	BDL
NY-S5	BDL	BDL	BDL	BDL
NY-S5	BDL	BDL	BDL	BDL
MEAN± STD	**0.303±0.130**	**1.251±0.500**		**0.383±0.050**
KAY-S1	BDL	BDL	BDL	BDL
KAY-S1	BDL	BDL	BDL	BDL
KAY-S2	BDL	BDL	BDL	BDL
KAY-S2	BDL	BDL	BDL	BDL
KAY-S3	BDL	1.0864	BDL	BDL
KAY-S3	BDL	0.61	BDL	BDL
KAY-S4	BDL	BDL	BDL	BDL
KAY-S4	BDL	BDL	BDL	BDL
KAY-S5	0.8164	BDL	BDL	BDL
KAY-S5	0.379	BDL	BDL	BDL
MEAN± STD	**0.597±0.310**	**0.848±0.330**		
BUG-S1	BDL	BDL	BDL	BDL
BUG-S1	BDL	BDL	BDL	BDL
BUG-S2	0.8	BDL	BDL	BDL

BUG-S2	0.399	BDL	BDL	BDL
BUG-S3	BDL	BDL	BDL	BDL
BUG-S3	BDL	BDL	BDL	BDL
BUG-S4	BDL	BDL	BDL	BDL
BUG-S4	BDL	BDL	BDL	BDL
BUG-S5	BDL	BDL	BDL	BDL
BUG-S5	BDL	BDL	BDL	BDL
MEAN± STD	**0.599±0.280**			

NY=Nyagatare, KAY= Kayonza, BUG= Bugesera, BDL= Below detection limit

IX.2 Pesticide residues in cabbages

Cabbages samples	Cypermethrin ppm	Abamectin ppm	Cyhalothrin ppm	Profenofos Ppm
NY-S1	BDL	BDL	BDL	BDL
NY-S1	BDL	BDL	BDL	BDL
NY-S2	BDL	BDL	BDL	BDL
NY-S2	BDL	BDL	BDL	BDL
NY-S3	0.924	BDL	BDL	1.398
NY-S3	0.422	BDL	BDL	0.731
NY-S4	BDL	BDL	BDL	BDL
NY-S4	BDL	BDL	BDL	BDL
NY-S5	BDL	BDL	BDL	BDL
NY-S5	BDL	BDL	BDL	BDL
MEAN±STD	**0.67±0.35**			**1.064±0.470**
KAY-S1	BDL	BDL	BDL	BDL
KAY-S1	BDL	BDL	BDL	BDL
KAY-S2	BDL	BDL	BDL	BDL
KAY-S2	BDL	BDL	BDL	BDL
KAY-S3	BDL	BDL	BDL	BDL
KAY-S3	BDL	BDL	BDL	BDL
KAY-S4	BDL	BDL	BDL	BDL

KAY-S4	BDL	BDL	BDL	BDL
KAY-S5	BDL	0.0709	BDL	BDL
KAY-S5	BDL	0.1569	BDL	BDL
MEAN±STD		**0.11±0.06**		
BUG-S1	BDL	BDL	BDL	BDL
BUG-S1	BDL	BDL	BDL	BDL
BUG-S2	0.3407	BDL	BDL	0.584
BUG-S2	0.1702	BDL	BDL	0.271
BUG-S3	BDL	BDL	0.493	BDL
BUG-S3	BDL	BDL	0.242	BDL
BUG-S4	BDL	BDL	BDL	BDL
BUG-S4	BDL	BDL	BDL	BDL
BUG-S5	BDL	BDL	BDL	BDL
BUG-S5	BDL	BDL	BDL	BDL
MEAN±STD	**0.25±0.12**		**0.364±0.170**	**0.427±0.220**

IX.3 Pesticide residues in oranges

Oranges samples	**Cypermethrin ppm**	**Abamectin ppm**	**Cyhalothrin ppm**	**Profenofos ppm**
NY-S1	BDL	BDL	BDL	BDL
NY-S1	BDL	BDL	BDL	BDL
NY-S2	0.252	BDL	BDL	BDL
NY-S2	0.141	BDL	BDL	BDL
NY-S3	BDL	BDL	BDL	BDL
NY-S3	BDL	BDL	BDL	BDL
NY-S4	BDL	BDL	BDL	BDL
NY-S4	BDL	BDL	BDL	BDL
NY-S5	BDL	BDL	BDL	BDL
NY-S5	BDL	BDL	BDL	BDL
MEAN±STD	**0.196±0.080**			
KAY-S1	ND	BDL	BDL	BDL
KAY-S1	ND	BDL	BDL	BDL

KAY-S2	BDL	BDL	BDL	BDL
KAY-S2	BDL	BDL	BDL	BDL
KAY-S3	BDL	1.21	BDL	BDL
KAY-S3	BDL	0.99	BDL	BDL
KAY-S4	BDL	BDL	BDL	BDL
KAY-S4	BDL	BDL	BDL	BDL
KAY-S5	BDL	0.63	BDL	BDL
KAY-S5	BDL	0.57	BDL	BDL
MEAN±STD		**0.833±0.380**		
BUG-S1	BDL	BDL	BDL	BDL
BUG-S1	BDL	BDL	BDL	BDL
BUG-S2	BDL	BDL	BDL	BDL
BUG-S2	BDL	BDL	BDL	BDL
BUG-S3	BDL	BDL	BDL	BDL
BUG-S3	BDL	BDL	BDL	BDL
BUG-S4	BDL	BDL	BDL	BDL
BUG-S4	BDL	BDL	BDL	BDL
BUG-S5	BDL	BDL	BDL	1.583
BUG-S5	BDL	BDL	BDL	0.86
MEAN±STD				**1.193±0.530**

APPENDIX. X.

Results of risk characterization index

Pesticide	ADI mg/kg bw /day (FAO/WHO)	Concentration levels found in this work mg.kg–1		Estimated Daily Intake (mg/kg bw/day)	HRI (Health Risk Index) HRI = EDI/ ADI
Cypermethrin	0.020	Tomato	0.599	3×10^{-4}	1.5×10^{-2}
		Cabbage	0.665	1.6×10^{-4}	8×10^{-3}
		Orange	0.196	1.8×10^{-5}	9×10^{-4}
Profenofos	0.030	Tomato	0.383	2×10^{-4}	6.6×10^{-3}
		Cabbage	1.064	2.5×10^{-4}	8.3×10^{-3}
		Orange	1.193	1.1×10^{-4}	3.6×10^{-4}
Abamectin	0.001	Tomato	1.251	6.4×10^{-4}	6.4×10^{-1}
		Cabbage	0.100	2.4×10^{-5}	2.4×10^{-2}
		Orange	0.833	1.4×10^{-5}	1.4×10^{-2}
Cyhalothrin	0.005	Tomato	-	-	-
		Cabbage	0.368	7.8×10^{-5}	1.56×10^{-2}
		Orange	-	-	-

APPENDIX. XI.

XI.1 (a) Farmer in spraying pesticides and (b) Tomato farming in Nyagatare

(a)  (b) 

XI.2. Most applied pesticides in Eastern Province (a) Mancozeb, (b) Abamectin and (c) Cypermethrin

(a) 

(b) 

(c) 

APPENDIX XII

XII.1. Tomato samples before extraction

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XII.2. Tomato sample in extraction process.

APPENDIX XIII.

Sample chromatogram