COLLEGE OF SCIENCE AND TECHNOLOGY

SCHOOL OF SCIENCE

DEPARTMENT OF CHEMISTRY

ASSESSMENT OF THE SELECTED PESTICIDE RESIDUES IN CABBAGE, EGGPLANT AND TOMATO FROM RUSIZI AND NYAMASHEKE DISTRICTS OF WESTERN PROVINCE OF RWANDA.

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

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DECLARATION AND COPYRIGHT

I, Ntamuturanyi Jean Bosco, hereby declare that, this masters dissertation entitled “assessment of the selected pesticide residues in cabbage, egg-plants and tomato from Rusizi and Nyamasheke districts western province of Rwanda” is my own orginal work. It is submitted at the University of Rwanda for partial fulfilment for the award of the degree of masters in Environmental Chemistry of the University of Rwanda. This dissertation has never been submitted elsewhere for anyother award of degree or academic certificate.

Student:

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We supervisors of this masters dissertation ,Confirm for its orginality and that it has been submitted for examination with our approval.

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DEDICATION

To

My Mother

My beloved wife , son and daughters

My brothers and sisters with love and respect

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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

Using pesticides in agriculture is an alternative taken for increasing crops production. However, the continuous use of these chemicals generates negative effects to the living health specifically to farmers and crop consumers. Due to the high availability of water and warmest weather, the Western Province of Rwanda is focusing among its agriculture targets, the increase of the production of vegetables and fruits. In order to reach this target, farmers from this region of Rwanda use a variety of insecticides to safeguard their crops against various pests. However, this poses a concern for environmental pollution in general as well as food and health contamination. Prior to advising farmers, consumers, and decision-makers, this study attempted to understand the types of pesticides used in Rwanda's Western Province and to evaluate the prevalence of pesticide residues in various crops.

The survey realized on 300 farmers and 30 agrodealers was conducted to get data on the types of pesticides used in this area and how they are handled by users. High Performance Liquid Chromatography was used to track the residue levels of selected agrochemicals in cabbage, tomato and eggplant samples which were randomly taken from different markets of this province.

The results indicated that Metalaxyl, Cypermethrin, Abamectin, Deltamethrin and Profenofos were the most applied pesticides used in this locality of Rwanda. Survey data indicated that 95 % of the interviewed farmers applied these chemicals using different illegal ways which risk to cause their intoxication and environmental pollution. Among these interviewed farmers, 96 % didn't get any training on the use of these chemicals while 93.4 % handled them without any protective equipment. These risk not only to not respect of mixing labels, but also to mismanagement of these agrochemicals. The results from chemical analysis showed that 30%; 36.67% and 23.33% of the analyzed tomato, cabbage and eggplant, respectively, were contaminated with pesticide residues. The most detected pesticides in all types of samples, tomato, cabbage and eggplant was Metalaxyl which detected at high level of 1.947ppm in tomato and cyhalothrin detected in eggplant with a concentration of 1.97 ppm. Through this study it was found that improvement of management and awareness on the use of pesticides is highly needed for farmers, pesticides sellers and vegetable consumers to avoid risk of health intoxication and environmental pollution. For further research, it is advised to carry out similar studies on other crop, environmental and biological samples.

Keywords: Pesticide residues, QUEChERS methods, Vegetables.

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LIST OF ABREVIATIONS

ABM: Abamectin

ACN: Acetonitrile

ADI: Accepted daily intake

BHC: Benze hexachloride

BLOQ: Below limit of quantification

BLD: Below of limit of detection

BUG: Bugarama

Bush: Bushenge

Cab: Cabbage

DDT: Dichloro-Diphenyl-Trichloro Ethane

EC: Emulsifiable Concentrate

Egpl:eggplant

EDI: Estimated daily intake

EDTA: Ethylene tetra acetic acid

EU: European Union

FDA: Food and Drugs Authority

GABA: Gamma amino butyric acid

GC: Gas Chromatography

GCB: Graphite carbon black

HPLC-RP: High Performance Liquid Chromatography-Reverse phase

IPCS: International Programme on Chemical Safety

LD: Lethal Dose

LC: Liquid chromatography

LOD: Limit of detection

LOQ: Limit of quantification

MRLs: Maximum Residue Levels

NYAM: NYAMASHEKE

TY: Tyazo

PPE: Personal protective equipment

PPM: Part per million

PPb: part per billion

REI: Restricted entry interval

S: Slope

Sd: Standard deviation

SSR: Spiked sample result

RSB: Rwanda Standard Board

T: Tomato

USR: Unspiked sample result

ICCM: International conference on chemical management

ISP: International Science Programme

SC: Suspension concentrate

SP: Soluble powder

Rpm: Revolution per minutes

QuEChERs: Quick Easy Cheap Effective Rugged and Safe

USD: United State Dollar

UV: Ultra-Violet

CHAPTER 1: GENERAL INTRODUCTION

1.1. Introduction

Rwanda like other developing countries is experiencing of an alarm population increasing with an annual population growth , the current population density of Rwanda in 2023 is 535.11 people per square kilometer [2]. Agriculture is one of the priorities fields of investment in order to achieve vision 2050, in parallel with environmental protection and sustainability [3]. However, the sector is facing with different challenges. The increase in the country population requires a concomitant positive change in food availabilty 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].

Any agrochemical or mixture of agrochemicals that are used to increase the production or to protect crops or foodstuff is referred to as pesticides [1]. The use of pesticides in agriculture implies that produced food may be contaminated by their residues at the level of becoming a food safety concern. Hence, the assessments of pesticide residues in tomato, cabbage and egg-plant cultivated in Rwanda Western Province was attempted in this study.

In farming, pesticide residues results a big word problems in environment and affects human's health. More than 1000 compounds may be used in agriculture for pest control and preventing undesirable weeds or insects [2].

Different legal frameworks such as codex and European Commission directives have been put in place to establish the residue level acceptable in safe foodstuff [3]. All foodstuff containing chemicals exceeding levels are questionable and should be avoided by consumers. The assessment of the residue levels of agrochemicals used to protect cabbage, tomato and egg-plant constitutes a a major subject to assure consumers and to advise both farmers and policy markers in the fields of agriculture and food proceecing.

1.2. Problem statement

The faster growth of population and high food demand require the use of chemical pesticides for food production [4]. Bugarama valley in Rusizi district has the lowest altitude equals to with 974 m and therefore it is considered as a hot area, farmers cultivate tomato, cabbage and eggplant as warm season crops [5]. Due to favorable climate conditions such as high temperature and water availability, Bugarama region is home to a variety of insects which are likely to damaging crops [6]. Hence, farmers use the pesticides for pest managements over their crops production.

Due to the wishes of selling good-looking vegetables and to the lack of the knowledge on appropriate use of pesticides, farmers spray a large amount of pesticides not even respecting the pre-harvesting intervals.These bad practices are ; exposing the consumers and environment to pesticide residues leading to serious public health risk. Therefore, this study has been conducted to assess the residues of selected pesticides in cabbage, eggplant and tomato from Rusizi and Nyamasheke districts in western province of Rwanda to advise both farmers and consumers.

1.3. Significance of the study

In Rwanda vegetable cultivation is practiced countrywide, it provides exceptional opportunities for farmers in the country through employment creation and income generation business, this is a unique area of economic development at rural level, national level. Farmers and other population consume a large number of vegetables [7]. Therefore, this sector should be kept safe and hence constant surveillance activities are required.

1.4. OBJECTIVES

The main objectives :

To determin residue levels of the most used pesticides in the farming of cabbage, tomato and eggplant produced in Rusizi and Nyamasheke districts of western province of Rwanda.

The Specific objective :

i. Identify the most used pesticides in growing cabbage, tomato and eggplant from Rusizi and Nyamasheke in Rwanda western province.
ii. Determine the levels of residues of the most used pesticides as revealed in specific objective i.

1.6. Study Area

The vegetables are grown in Rwanda western province where there is water availability and high temperature, this area there is warming 24.6 ℃. Eggplant are grown in western, southern and Eastern Provinces, the warmest area, in western province more than 85% of carrots as vegetable are produced in Western Province [8]. Following cabbage in terms of volume produced in terms and area farmed, tomato is the most valuable vegetable in Rwanda [8]. Bugarama and other area near by Kivu Lake are hot area and there is water availability, these areas cultivate a higher amount of vegetables (tomato, eggplant and cabbage) so farmers use a huge amounts of pesticides for pest control for better production. This area also is on the border of country where fraudulent pesticides can be entered and should be used by farmers. This study will cover the vegetable samples from Rusizi district (Bugarama Valley) and Nyamasheke district (Nyabitekeri,Bushenge,kagano,Bushekeri and Macuba sectors) samples from nearby Kivu Laker. In Rusizi district; samples have been sampled at Bugarama market, Nyamasheke samples have collected at Bushenge market and Tyazo market.

CHAPTER 2: LITERATURE REVIEW

2.1. Pesticide use

The use of pesticides by humankind dates back to thousands of years ago, different compounds such as sulfur, mercury and arsenic containing substances were involved in the control of insects, body lice and many other pests [9].

2.2. Classification of pesticides

Pesticides can be classified as by target organism, chemical structural and physical properties state [10, 11]. They can also be classified as inorganic, synthetic and biological pesticides (which include micro biopesticides and biochemical pesticides). Basically, pesticides are classified into five categories depending to target pest organism:

(i) Insecticides which attack the nervous system of the pests. Breakdown the nervous system of the pests and helps gain a rapid inhibition. For examples**:** organo-phosphate malathion, diazonon, chlorpyriphos; carbamate (carbaryl,methomyl, propoxur) ; biological insecticide: pyrethrin [12].

(ii) Herbicides that are used to inhibit the growth of weeds. These products disturb the conductive conditions for reactions that are responsible for the growth of the plant for examples: Metolachlor or atrazine, cyanazine2,4-D, Gyphosate.

(iii) Fungicides that act either by limiting the development of fungi or by eliminating fungi completely.

(iv) Ronticides which are chemicals used to control mice, rats and other rodents. Chemicals which control other mammal, beds and fish are also grouped here according to regulatory agency for examples: mancozeb, copper sulfate, folpet, captan, …

(v) Fumigants those are pesticides which produce gas to soil and plant for pest,including nematodes, fungi, bacteria, insects and weeds. For examples: methyl bromide, chloropicrin., methyldichloropropane, propylene oxide, dibromo-chloropropane [13].

2.3. Class of pesticides based on their toxicity

Pesticides classiffication based on toxicity 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.

The world health organization classify pesticide into five class [21].

Table 1:The table below indicate the different class of pesticides and their lethal dose.

trClassification	The rat LD in mg/ kg body weight	
	Oral route of entry	Dermal route of entry
Ia Extremely toxic	< 5	Less 50
Ib Highly hazardous	< 5 - 50	50-200
II Moderately hazardous (moderately toxic)	Greater than 50-500	200-2000
III Slightly toxic	Greater than 2000	Over 20000
V Non toxic (unlikely for presenting acute hazardous).	>5000 higher	

1. 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 [14, 15]

2. Class II of highly hazardous pesticides (Ib)

This class 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.

3. The third category of moderately hazardous pesticide (II)

This class 3 contains the products with LD_{50} of oral exposure between 50 and 500 mg/kg Examples of chemicals classified in this group are; Bifenthrin, Butylamine, Chlorpyrifos, Copper sulfate, Deltamethrin, Dimethoate, Pyrethrins [16, 17] .ty

4. **The fourth group of slightly hazardous pesticides (III)**

The class four has LD_{50} between 500 and 5000 mg/kg and contains the products like; Acephate, Copper hydroxide, Amitraz, Hexazinone, Metalaxyl, Pyrifenox [16, 17].

5. **The fifth category is the unlikely to present acute hazardous (nontoxic)**

Nontoxic pesticides are 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 [18, 19].

2.4. Generality on the concerned pesticides

2.4.1. Metalaxyl

The Epa released metalaxy registration standards In June 1981(NTS # PB 82 History 172297) and September 1988 (NTIS PB89-128979) after the pesticide was registered in the United States in 1979.

The plant diseases caused by fungi specialy omycet and woter mold fungi metalaxylpesticide is used tonctrol these fungi, Metalaxy (figure 4) is used to feed crops on many foods and non-food [20].

Metalaxyl may be applied by surface spraying, foliar application, drip irrigation or sprinker, soil mix or seed treatment.it is used with particular limitation on occurrent product labeling prohibit use of treated seed for feed, food or oil; feeding clippings, crop waste or vines to livestock; or grazing treated areas. Preharvest interval restrictions also apply [20].

People may be expose to this metalalaxyl pesticide during application agricultural activities or after and in other contests.

Metalaxyl in normal environmental conditions is moderately stable. Once is exposed to the sun light, it is photolytically stable in water and its has a half-life of 400 days, and is stable to photodegradation in soil. Metalaxyl has a moderately rapid degradation in aquatic systems, and its has very little of the chemical lost to volatilization.

Metalaxyl and its main components are rapidly leach in many soils,and metalaxyl is persistent and mobile .The potential for ground water contamination of metalaxyl and its main degradate is shown by monitoring data.

Metalaxyl used in differents activities may be exposed to non target organism and may results negative effect to human health like a eye irritanta s it has been classified as a Group E carcinogen [21].

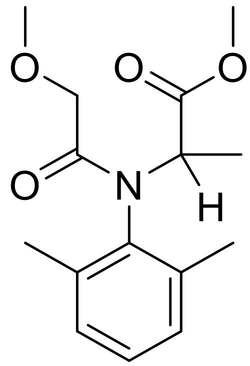

Figure 1: Chemical structure of metalaxyl

2.4.2. Cyhalothrin

Cyhalothrin (figure 5) has the active ingrendient which is very low soluble in water and makind emulsion when diluted.

Cyhalothrin is one of the most applied insecticides used kill the insect pests like; ants, termites, cockroaches, spiders, earwigs, bees, bed, bugs, scorpions, silverfish, wasps, and ticks. Cyhalothrin is a pyrethroid insecticide [15].

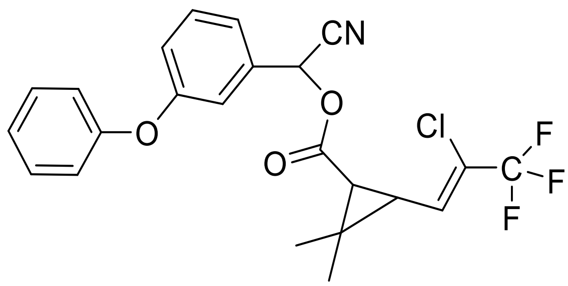

Figure 2: Chemical structure of cyhalothrin

Cyhalothrin as other Pyrethroids compounds disrupt the normal functioning of insect nervous system by delaying closure the sodium channel. Cyhalothrin affects neuromuscular system of non target and target organism by disrupting the normal system. After this disrupting, cyhalothrin may influence insect paralysis or death [22]. 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 [22].

2.4.3. Alpha-cypermethrin

Alpha -cypermethrin (figure 6) is used for controlling a wide range of insects in fruits, in vegetables, cereals and other crops [23]. The repeated dose of alpha cypermethrin reduces body weigth gain, food consumption and induces signs of neurotoxicity the proof studies were conducted on rodents [23].

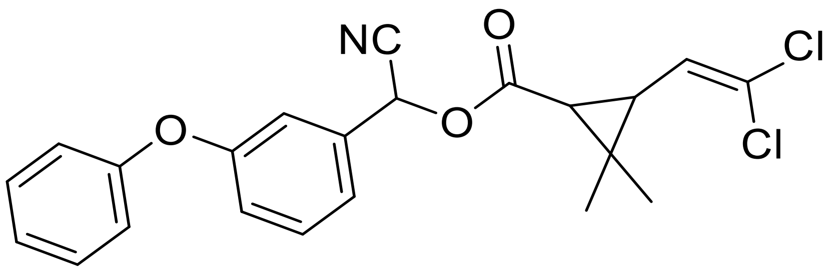

Figure 3 : Chemical structure of alpha-cypermethrin

2.4.4. Profenofos

Profenofos (figure 7) is a liquid with garlic order and a pale yellow amber color and its is an organo phosphate insecticide. WHO in 2018 recommended the current of acceptable current acceptable daily 0-0.03m =g/ kg body weight and its acceptable reference dose of 1 mg / kg bw. The mechanism of action of Profenofos is via inhibition of the acetylcholinesterase enzymes as other organophosphate pesticides .

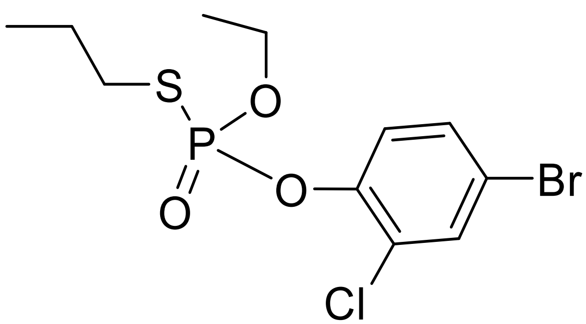

Figure 4: Chemical structure of profenofos

This pesticide is a neurotoxin acting as a cholinesterase inhibitor in insect nervous system [24, 25, 26]. It is the highest pesticide residues, moderately hazardous, toxic to human reproductive system by contributing to low sperm, reduced neurobehavioral performance and learning deficits.

2.4.5. Abamectin

Abamectin (figure 8) contains two substances mixed together avermectin containing B1a 80% and avermectin B1b 20% these two components have similar toxicological and biological properties [27]. EPA classify abamectin as class II toxicity pesticide, a necessity of further hazard evaluation of avermectin is considerated by international regulatory agencies is strongly recommended [28].

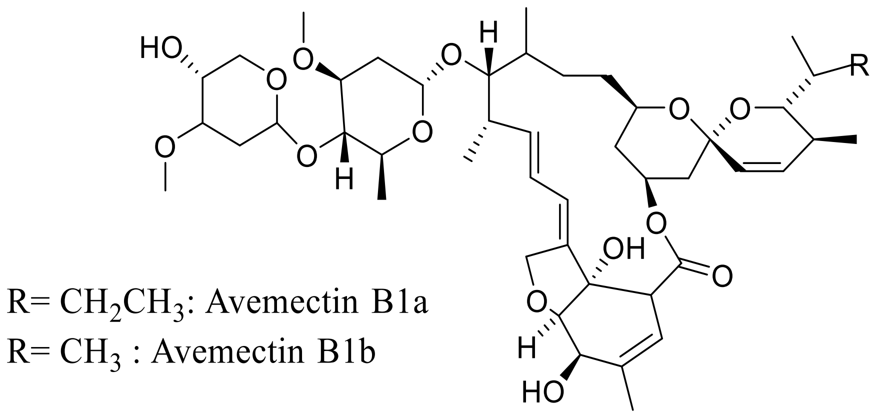

Figure 5: Chemical structure of abamectin

2.4.6. Chlorothalonil

Chlorothalonil (figure 9) acts firstly as mildewcides and fungicides and secondly has bactericide,algaecide,microbicides,insecticides and acaricide activities. Chlorothalonil is an acute toxicity category IV for the oral route of exposure and in toxicity category II for the inhalation route. For acute dermal effects and acute skin irritation ,chlorothalonil is in Toxicity Category IV. Chlorothalonil produces severe eye irritation in rabbits (Toxicity Category I). The Agency has classified chlorothalonil as a likely human carcinogen [29].

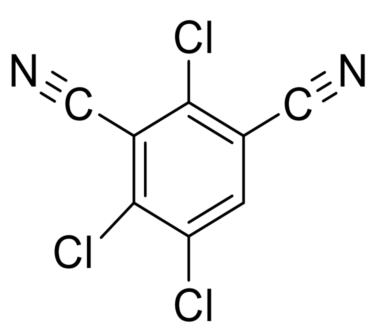

Figure 6: Chemical structure of chlorothalonil

2.4.7. Deltamethrin

Deltametrin (figure 10) acts as a pyrethroid ester insecticide in agriculture its is an inhibitor of a calcium channel agonist and an antifeedant. It is an aromatic ether, an organobromine compound, a nitrile and a cyclopropane carboxylate ester. The direct contact of deltamethrin on insect result the insect to die or insect die once eat deltamethrin . Deltamethrin disrupts the normal nervous system function of insects.

It is less toxicto mammals due to their higher body temperature, larger body size, and decreased sensitivity to the chemical [30].

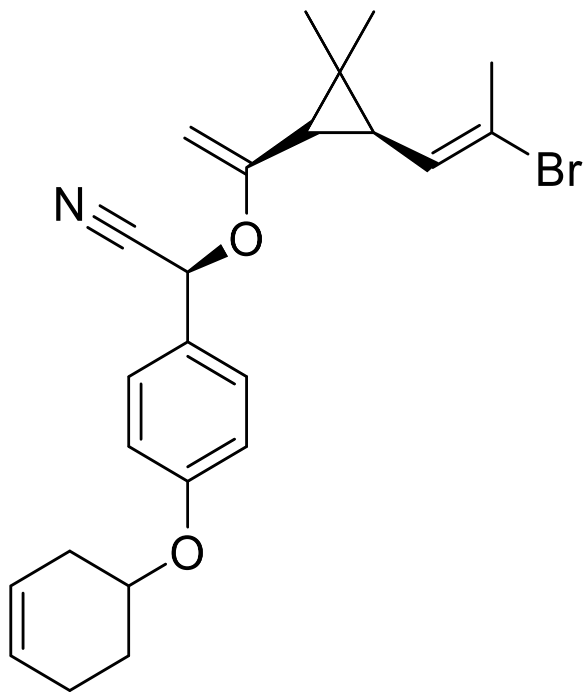

Figure 7: Chemical structure of deltamethrin

CHAPTER 3 : MATERIALS AND METHODS

3.1. Data collection

The survey was used to know the 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 of some parts of the Westen Province of Rwanda. The number of populations interviewed were obtained based on statistical formula for sampling. Assume standard of a 95 % confidence $n = \frac{N}{1+N\,(e)^{\wedge}2}$, Taro Yamane formula (Yamane 1973) [31, 32] **equation 1.**

With:

n is the number of surveys needed (sample size)

N: Number of Farmers who cultivate vegetables in sampling area.

e: error (0.05) reliability level 95 % or e is level of precision always set to the value of 0.05

The number of population living in the targeted area, Bugarama, Shangi, Bushenge, Nyabitekeri, Kanjongo, Bushekeri and Kagano sectors, frequenting the Bugarama, Bushenge and Tyazo markets, was estimated to 212,836 [33]. As 72% of Rwandan population are practicising farming activities, a number of 153,242 was taken as being farmers. Considering that 1.23% are growing vegetables in that area, the number of farmers for vegetables was estimated to1885 [34].

Therefore, the significant number of people to be interviewed was calculated as

$n = \frac{1885}{1+1885\,(0.05)^{\wedge}2}$ = 329.99 = 330.

A survey was conducted using a questionnaire (Appendix I) which was developed according to the study objectives.

3.2. Number of samples and sampling

A total 90 samples have been collected for pesticide residues analysis. The vegetable samples including 30 samples of tomato coming from 2 districts in 3 different markets; 30 samples of cabbage and 30 samples of eggplant.

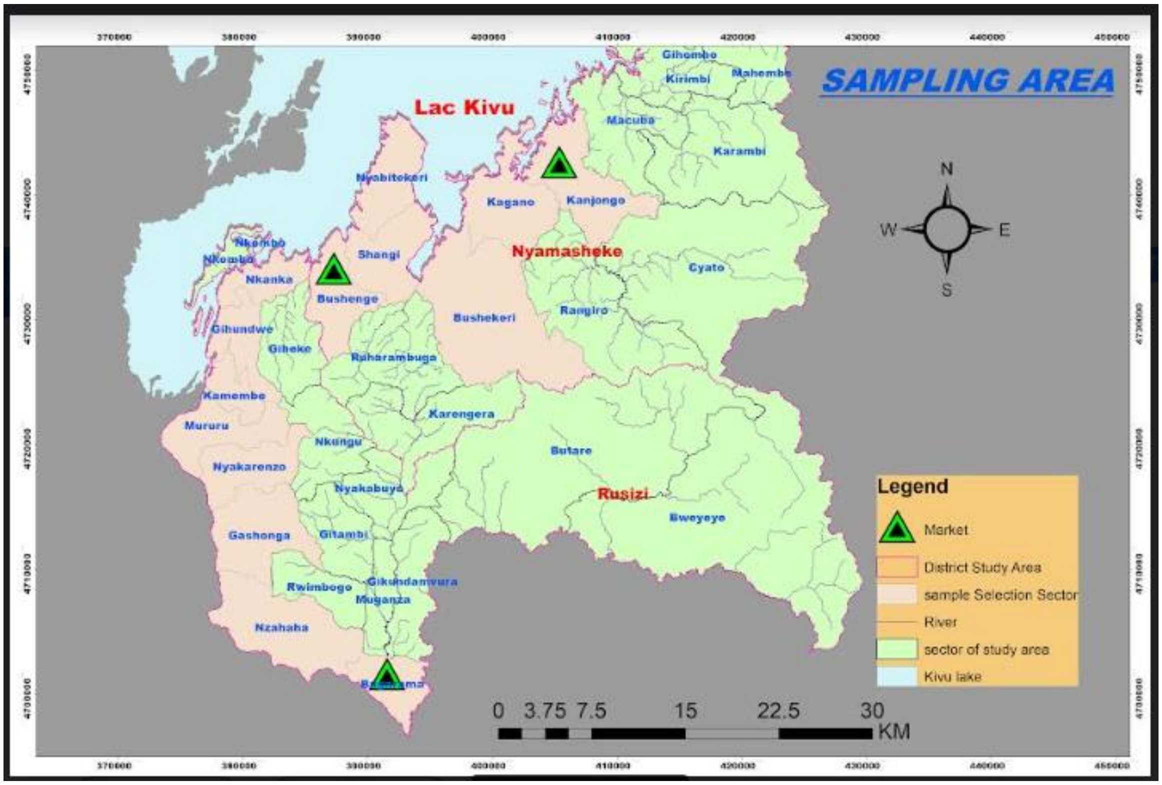

Figure 8: Map of sampling area

Frequency of sampling

Each district market has been sampled three times in different days.

Each representative tomato, cabbage or eggplant sample is composed by composite 2 to 3 samples of the same vegetable collected through random sampling.

Tomato samples, cabbage and eggplant samples would be collected separately.

During sampling the gloves have been used and changed every time before collecting the next sample.

Ninety samples of cabbage, eggplant and tomato were collected in three markets of western province (samples from Bugaramavalley ,Bugarama market In Rusizi District) samples from Nyabitekeri-Bushenge and Shangi sector (Bushenge Market) and samples from Kanjongo-Bushekeri-Macuba (Tyazo market) in Nyamasheke district.

In these ninety samples collected there were 30 samples of tomato, 30 samples of eggplant and 30 samples of cabbage from western province of Rwanda.

Tomato sample 1 (T1) to sample 10 (T10) are samples collected from Bugarama market.

Tomato Sample eleven (T11) to sample 20 (T20) are tomato samples collected from Bushenge Market

Tomato sample 21 (T21) to sample 30 (T30) are sample collected from Tyazo market

Cabbage sample 1 (cab1) to sample 10 (cab 10) samples from Bugarama S11 to 20 collected from Bushenge market and Sample 21 to 30 cabbage samples collected at Tyazo market.

The dust from samples were removed without washing and then the sample have been placed into the collection bags specially polyethylene bag.

All the samples were placed in the polyethylene bag contained ice to avoid contamination and deterioration, labelled and closed separately and transported to the laboratory and kept at -20 °C until analysis.

Storage: Laboratory samples which were not analyzed immediately have been stored under conditions that minimize decay.

Fresh sample have stored in the refrigerator but no longer than five days.

The dried product should be kept at room temperature, and its stored time is expected to exceed two weeks.

Each market sample will be separately washed using tap water for cleaning them and then homogenized to obtain a uniform sample.

3.3. Extraction of pesticide residues from vegetable samples

Samples were reduced into pieces and then grinded using a blender or mortar in order to obtain a homogeneous sample extraction of pesticide residues from crops vegetable and fruit samples was done Using the QuEChERs Methods **(**Q-sep QuEChERS tubes easily remove matrix interferences**):**

QuEChERs method is a Quick Easy Cheap Effective Rugged and Safe multiresidues analytical approach. this technique involves liquid partitioning using acetonitrile and purifying the extract using dispersive solid-phase extraction [35].

QuEChERS is a novel sample preparation methodology for pesticide residue analysis that was developed between 2000 and 2002 and first reported in 2003 [36].

This method has already accepted by the international community of pesticide residue analysis and there are many publications of on pesticide residues in food performed with this method.

This method involves initially extraction with acetonitrile then partitioning step after adding of the mixture of salts.

An aliquot of the raw extract is cleaned up by dispersive solid phase extraction (DSPE).

The final extract is acetonitrile and is directly amenable to determinative analysis on Liquid chromatography and / or Gas chromatography

The QuEChErs method covers a wide analyte scope, including high polar pesticides as well as highly basic and acidic pesticides [37].

Each QUEChERS's q-sep tube contains:

Magnesium sulphate $MgSO_4$ which remove excess water in the analyte

PSA **(**primary and secondary amine) remove sugars, fat acids, pigments organic acids, anthocyanin pigments

GCB (graphite carbon black) remove pigments, sterols, nonpolar interference or C18-EC (end-caped) remove nonpolar interferences [38].

With QuECHERs method, a sample will be homogenized and undergoes a quick extraction step where the analyte is moving into organic solvent by the partitioning power of blend salt

After sample extraction, the sample will be cleaned up through the use of dispersive solid phase extraction (dSPE) the step that is also quick and easy to perform [38].

Procedures

Prepare Samples for LC or GC Analysis in Two Simple Stage

Analytes of interest are extracted from the sample through the addition of an organic solvent and a blend of salts. The salts enhance extraction efficiency and allow the normally miscible organic solvent to separate from the water in the sample

Stage1: Sample Extraction

The sample will be blended and weight 10 g of a homogenized sample

Add 10 mL of a mixture of 1 % acetic acid and acetonitrile, then shake vigorously for 1min and centrifuge 5 min at 1500 rpm

Add extraction salts and shake, then centrifuge to separate the phases centrifuge 5min at 10,000 rpm.

Stage 2: Sample Cleanup

Transfer supernatant to dispersive tube. Shake, centrifuge, and transfer to an autosampler vial for analysis by LC

Crush / Grind a vegetable sample using a blender/ Morton

Weigh 10 g of crushed and homogenized sample

Add 10 mL of a mixture of 1 % acetic acid and acetonitrile

Shake vigorously for 1 min and centrifuge 5min second at 5000 rpm

Sample extraction

Add extraction salts and shake (NaCl, MSO_4, PSA, CG)

Centrifuge to separate the phase 30 sec at 1500 rpm

Transfer 5 mL to dispersive tube

Sample clean up

Shake and centrifuge 5 min at 10,000 rpm → Inject 10 µL in HPLC system.

Figure 9: Method flow chart

Once the QUEChERS Q-Sep tube are not available, the following procedure should be followed related to QUEChERS method**:**

Weight 10 g of sample well chopped and Homogenized sample into a 40-50 mL tube of polypropene. Addition 10 mL of acetonitrile and shaking vigorously for 1min. Addition of 4 g anhydrous magnesium sulphate and 1g of NaCl followed by intense agitation.The whole sample is shaken for 30 sec. After take 1 mL aliquot of upper acetonitrile layer and transfer it into a centrifuge via 25 mg of primary and secondary amine PSA (Sorbent) and 150 mg of anhydrous $MgSO_4$ then the sample is shaken by hand or with the vortex mixer for 30 sec and centrifuged.The obtained supernatant is taken from the centrifuge and can be analyzed by Liquid Chromatography [39].

3.4. Instrument conditions

HPLC analysis was used for sample analysis.

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

A mixture of water and acetonitrile was used as mobile phase 60 mL of ACN into 40 mL of water for removal of any impurity, bubbles and dissolved oxygen.

The double distilled water and acetonitrile should be first filtered through 0.45 filter paper then sonicated for 15 min at the flow rate 1 mL per min at the temperature of 35 ℃.

Before analysis the sample;the sample of vegetable the HPLC method should had been validated using chromatographic parameters including recovery, reproducibility and limits of detection (LOD).

3.5. Quality control of analysis method

The analysis of pesticides has been performed using different methods as validation in order to check if the purpose of the methods used is fitting and if is achieved.

Different validation parameters were used to confirm validation**.**

3.5.1. Percentage recovery

This procedure was conducted by using the spiking of samples with two different concentrations in egg-plants, tomato and cabbage. The reference sample spiked at 2 levels (0.5 ppm and 0.1 ppm) of the targeted pesticide and pass through the same procedure as the sample

Calculation of percentage of recovery is fallow:

$Percentage\ of\ Recovery = \frac{\text{SSR} - \text{USR}}{\text{SSR}}\ x\ 100$ **Equation 2**: Recovery

Where SSR: spiked sample result and USR: Unspiked sample result.

3.5.2. Limit of Detection (LOD)

The Limit of detection LOD was determined by using calibration standards.

Limit of detection was calculated as per International Chemical Harmonization (ICH) guidelines as shown in equation below [40].

$LOD = \frac{3.3 \text{ x SD}}{\text{S}}$ **Equation 3**: Limit of detection

Where sd : is standard deviation of peak area of calibration curve

S: is the slope of calibration curve.

3.5.3. Limit of Quantitation (LOQ)

LOQ was determined by using calibration standards as LOD.

Limit of quantification can be calculated as per ICH guidelines using following equation, $LOQ = \frac{10 \text{ x SD}}{\text{S}}$ **Equation 4**: Limit of quantification

where, SD is the standard deviation of the peak areas of the analyte and S is the slope of the corresponding calibration curve [40].

Where LOD = is limit of detection.

3.5.4. Precision

We have calculated the precision by using different levels of concentrations of prepared pesticides from independents solutions.

Different levels of pesticides standard conditions were prepared in three different times in a day and the intra variation were studied and this is done in three different days for best determination of interday precision.

The precision is determined and expressed as percentage relative standard deviation (% RSD).

The relative standard deviation has been calculated from three replicates of each individual pesticide.

$RSD = \frac{\text{SD}}{\text{mean}}\ X100$ **Equation 5** : Relative standard deviation calculation

SD is the standard deviation and Mean of Peak area.

Data Analysis

The data were analyzed by descriptive statistics and other variety of analysis like statistical mean and standard deviation

The fields data analysis from survey were analyzed by using descriptive statistics Laboratory data were analyzed by using statistical mean and standard deviation.

CHAPTER 4: RESULTS AND DISCUSSIONS

Analysis of pesticide residues namely metalaxyl, deltamethrin, chlorothalonil, abamectin**,** cyhalothrin, alpha-cypermetrhine and profenofos in three vegetables viz tomato; eggplant and cabbage were performed.The results are presented and dicussed in this chapter.

The validation methods quality control like limit of quantification, percentage of recovery and precision detection were determined in this chapter.

4.1. Most used pesticides as indicated by the data collected from farmers.

The farmers were interviewed about how they use pesticides in their different vegetables and crops like, tomato maize, beans, cabbage, eggplant, irish-patatoes, oranges and other crops.

The results showed that, in Nyamasheke and Rusizi districts fungicides, insecticides, herbicides and acaricides are applied at 60%, 33%, 3% and 2%, respectively.

Metalaxyl (24%), chlorothalonil (13%) are the most applied fungicides in this region of Rwanda, insecticides applied in this province of Rwanda are dominated by cypermethrin (29.41%), profenofos and deltamethrin (21.16%) and abamectin (11.11%).

Data from survey showed that 95 % of the interviewed farmers applied these chemicals and among these interviewed farmers, 96 % didn't get any training on the use of these pesticides and 93.4 % handled them without any protective equipment.

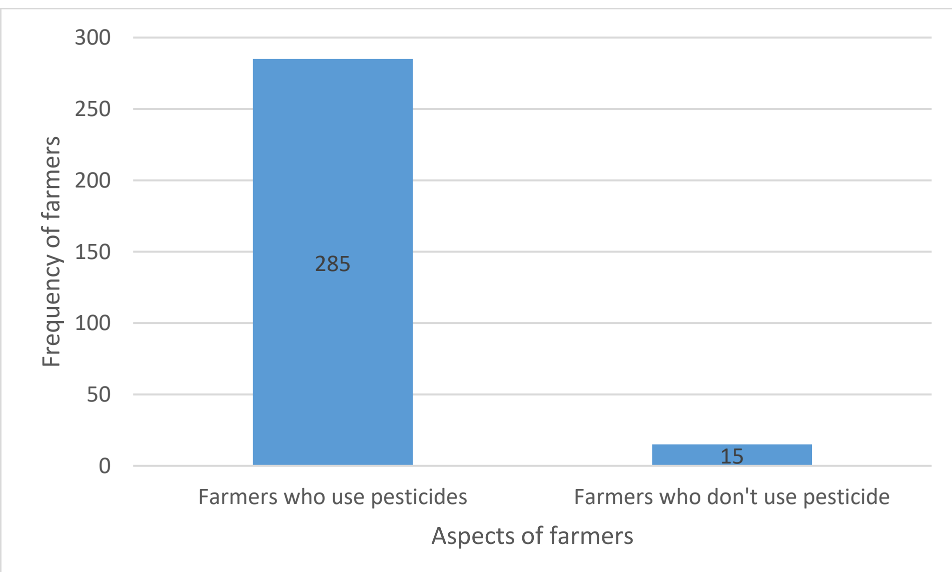

Figure 10:Farmers who use pesticides

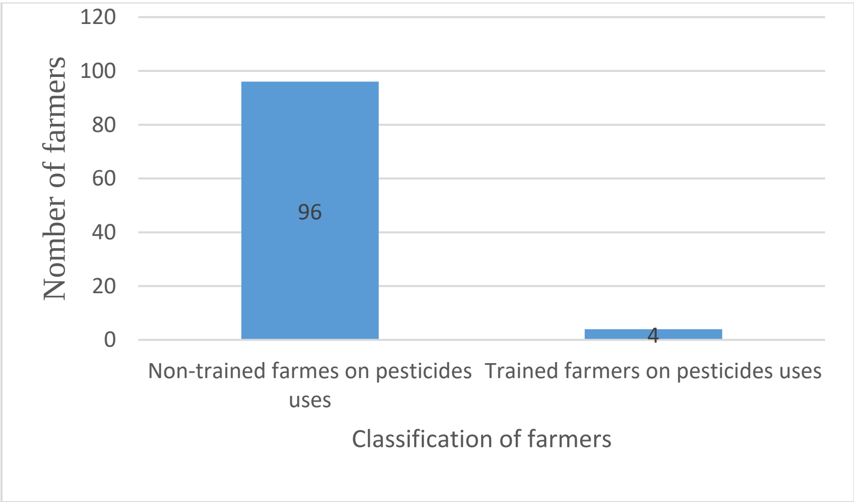

Figure 11:Trained and non trained farmers on the uses of pesticides

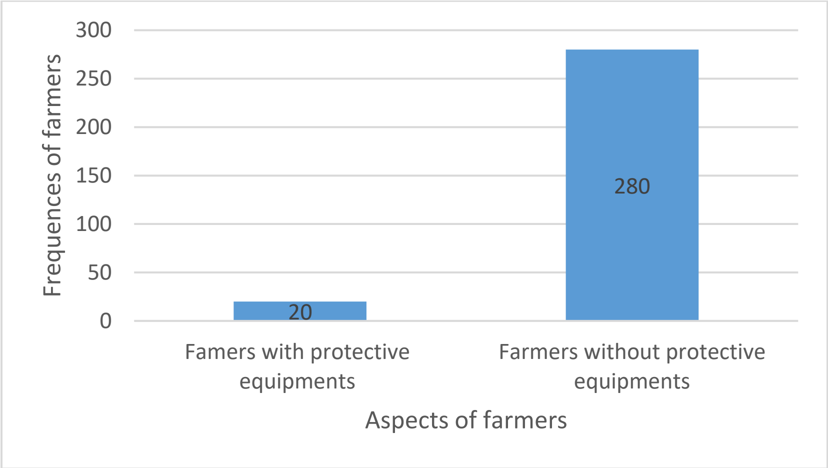

Figure 12:Farmers and protective equipments .

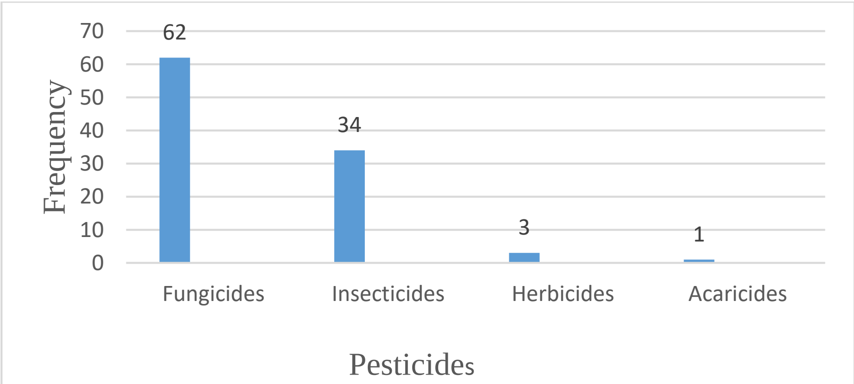

Figure 13: Classification of pesticides based on target organism

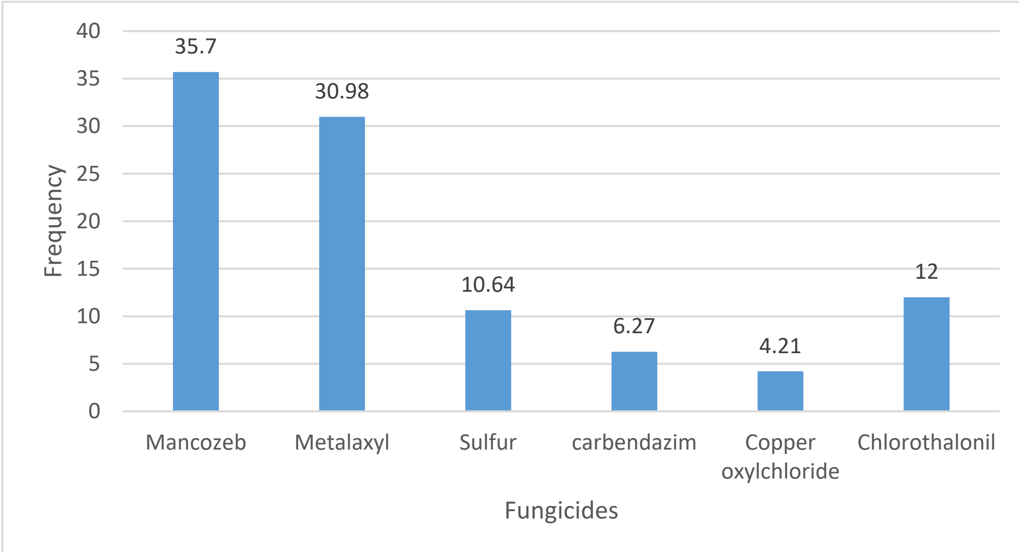

Figure 14: Most fungicides used in western province of Rwanda

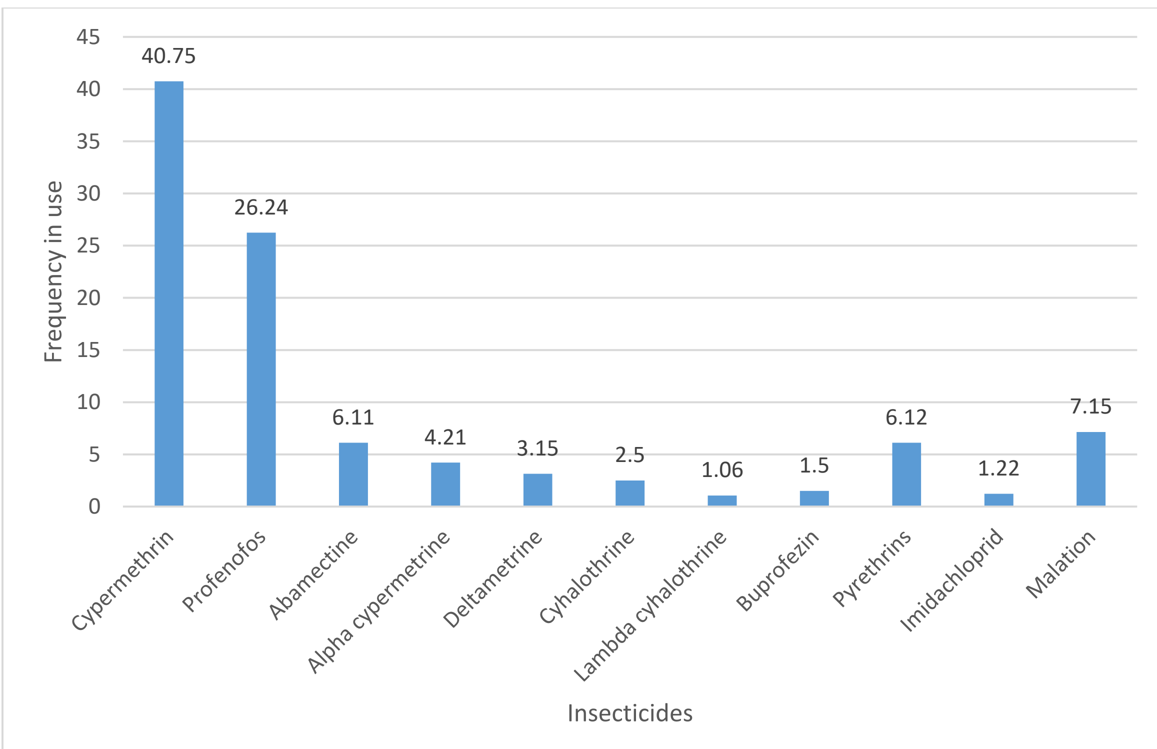

Figure 15: Most insecticides used in western Province of Rwanda

4.2. Detection of pesticide residues and quality control

Pesticide residues from tomato cabbage and eggplant of Rusizi and Nyamasheke disticts of the western province of Rwanda

The range of detection limit is between 0.00473 ppb and 0.059 ppb with alpha cypermethrin has a lowest detection limit and profenofos a highest detection limits the limit of quantification ranged between 0.032 ppb and 0.195 ppb (Appendix. IV**).**

The analyzed pesticide residues (methalaxyl, chlorothalonil, alphacypermethrin, profenofos, deltamethrin, abamectin and cyhalothrin) showed a good linearity with the correlation between 0.999 and 1.

The results prove that the method was accurate and rapid to determine the level of pesticides metalaxyl, chlorothalonil, alpha cypermethrin, profenofos, deltamethrin, abamectin and cyhalothrin) residues in tomato eggplant and cabbage.

Acetonitrile was used solvent and showed a good extraction efficiency for 7 selected pesticides.

The QuEChERS method was efficiency for all pesticide residues extraction. The same results were found by W. Xie et al. (2006) and O. Akoto (2015) [15], [41, 42, 43].

All calibration curves of analyzed seven pesticides showed a correlation coefficient more than 0.9998 confirm the best linearity of the method used.

The quantification and detection limits of seven analyzed pesticides were below their maximum residue limits this prove the efficacity of methods used in determination of pesticide residues in three vegetables. 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.

4.2. The presence of the pesticide residues in analyzed vegetable

The results of pesticide residues determined are presented (in appendixes IV) for tomato, cabbage and eggplant, respectively.

Before analysis, the used standards were injected in HPLC and their peaks recorded as references for further analysis of the extracts (pesticide residues from samples) **(**appendix V).

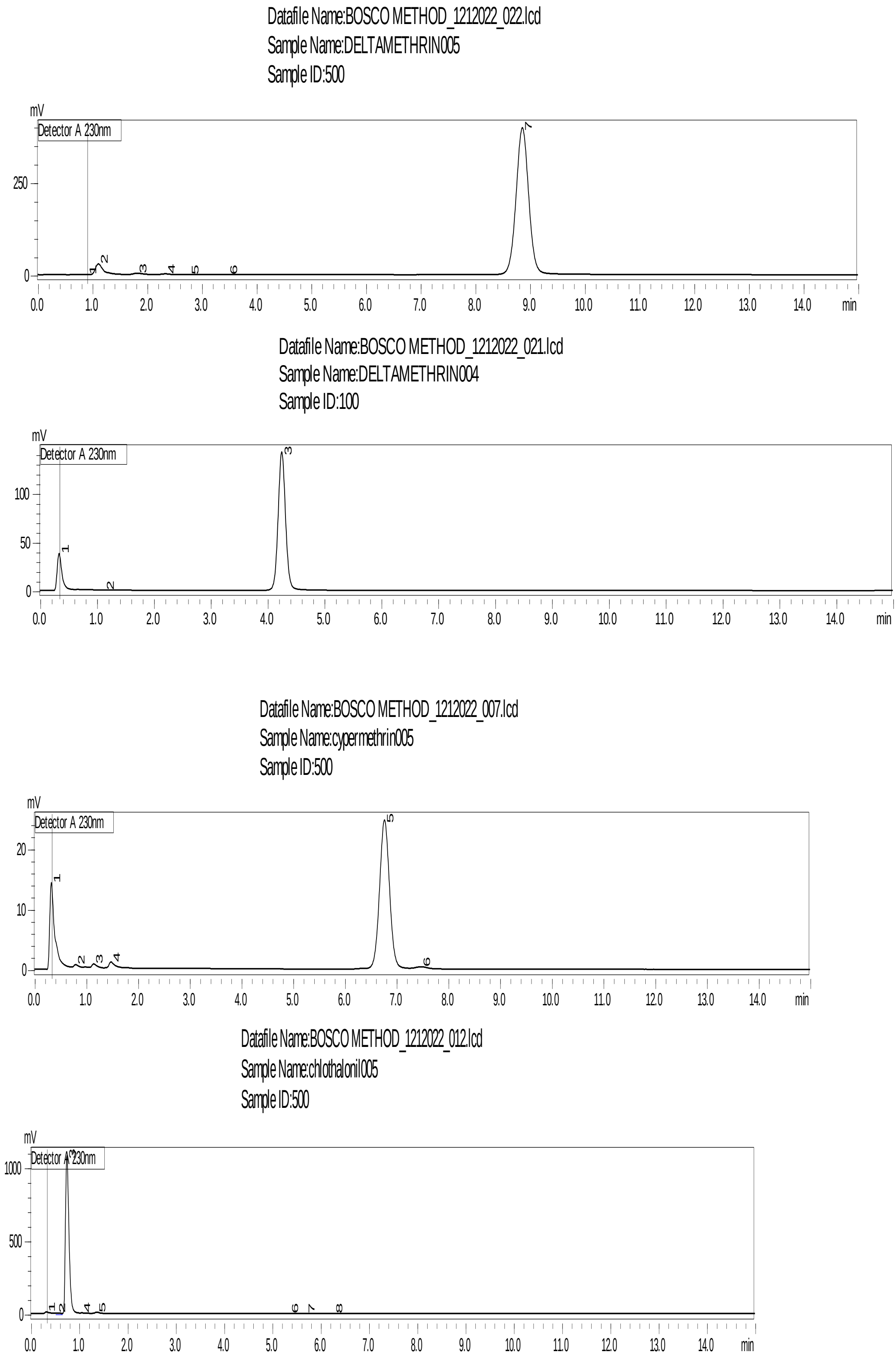

Figure 16 : Chromatograms for pesticide standards

4.2.1. Pesticide residues in analyzed tomato

In 30 samples of tomato analyzed only 9 (30%) samples presented pesticide residues and 21 samples (70%) of tomato contained no detectable values of pesticide residues.

Five pesticides (metalaxyl, deltamethrin, chlorothalonil, alpha cypermethrin.) are detected in tomato samples.

The most detected pesticide in tomato samples was metalaxyl in four samples (13.33%) the second one is chlorothalonil which was detected in two samples (6.66%); alpha cypermethrin, cyhalothrin, profenofos were detected in one sample (3.33%).

Abamectin and deltamethrin pesticide residues were not detected in tomato samples.

Two pesticides (metalaxyl, chlorothalonil) were detected at the same time in two samples also metalaxy and profenofos were detected at the same time in one sample. In nine samples detected pesticide residues 8 (88.88% of contaminated samples) of them are sample collected from Bugarama market and one detected is a sample collected from Bushenge market.

Methalaxyl was detected as the high value of pesticide residue in tomato T3 (1.97 ppm) sample taken from Bugarama market.

The presence of these pesticide residues in analyzed vegetable sample is related to the use of these chemicals by farmers for pests control and try increasing cropsproduction. The samples from Bugarama indicate the higher amount of pesticide residues due to excess in use of these pesticides also this area is warmest contains higher amounts of pests so farmers try to use a higher amounts of these pesticides.

Spraying these pesticides and mixing labelling and harversting intervals are not respected by farmers.

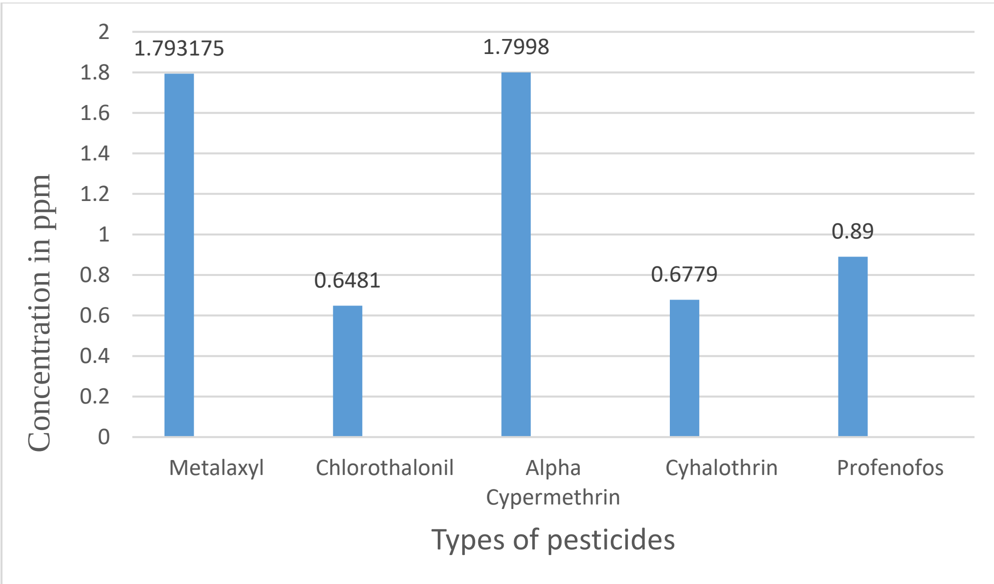

Figure 17: Mean of pesticide residues in tomato samples

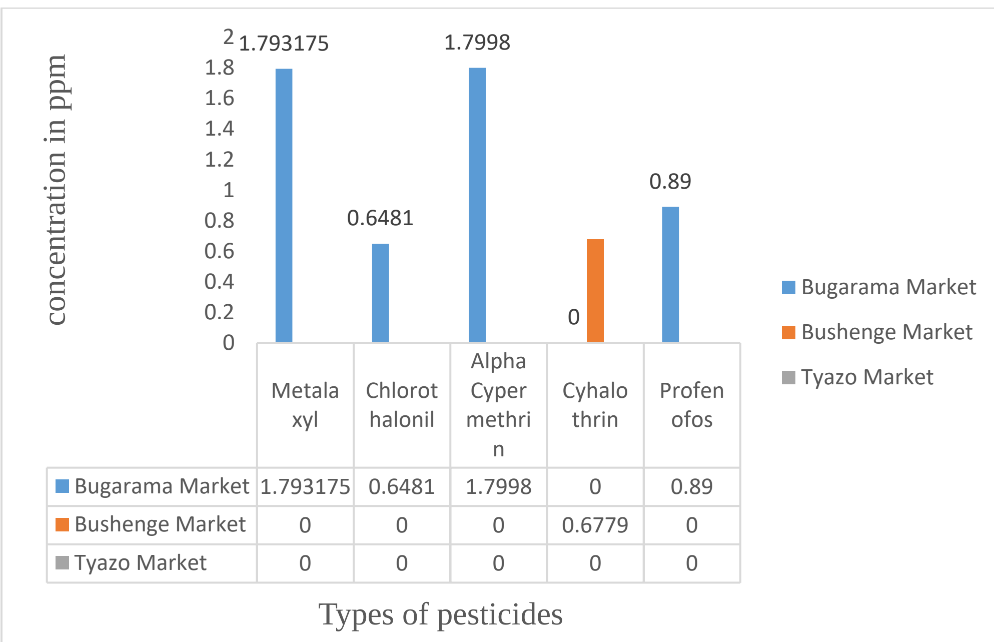

Figure 18: Mean of pesticide residues in tomato samples from 3 markets

The results obtained prove that some pesticide residue values are lower than thoe value indicated by international food regulators (chlorothalonil 0.64 mg/ kg < 5 mg / kg) and other values obtaine are higher than the maximum residues limits indicate by international food regulators [44].

4.3.2. Pesticide residues in analyzed cabbage

Two pesticides (metalaxyl and abamectin) were detected at the same time in one sample (cab 4) from Bugarama market and also alpha cypermethrin and abamectin were detected at the same time in one sample (cab 10) taken from Bugarama valley.

The presence of pesticide residues was detected in all market samples.

Abamectin pesticide residue was detected at the high value of pesticide residue in cabbage (1.684 ppm) sample taken at Bugarama market and the second high value is also abamectin (1.620 ppm) sample taken from Bugarama market.

Bugarama market samples indicated the presence of 3 pesticides (metalaxyl, alpha- cypermethrin and abamectin) in cabbage samples.

Bushenge market cabbage samples indicated the presence of 4 pesticide residues (metalaxyl, deltamethrin, alpha-cypermethrin and abamectin).

Tyazo market cabbage samples indicated the presence of two pesticide residues (chlorothalonil and deltamethrin).

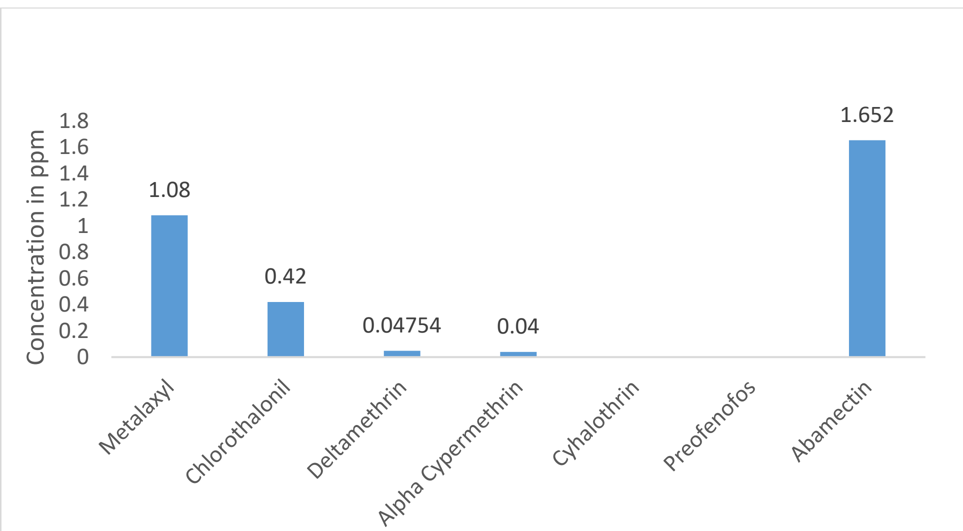

Figure 19: Mean of pesticide residues in cabbage samples

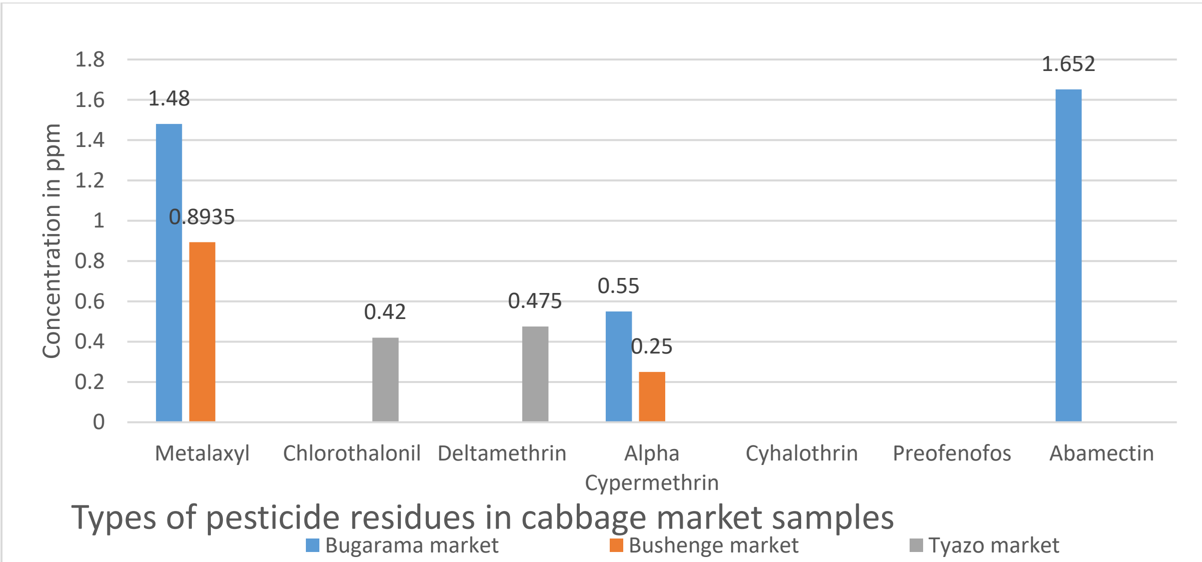

Figure 20: Mean of pesticide residues in cabbage samples from 3 markets

4.3.3. Pesticide residues in analyzed eggplant

Among 30 samples of eggplant analyzed six of them presented pesticide residues (20%) and in 24 eggplant samples the presence of pesticide residues were not detected (80%).

Five pesticides presented (metalaxyl, alpha-cypermethrin, deltamethrin, cyhalothrin and abamectin) ; chlorothalonil, and profenofos were not detected in eggplant samples.

Metalaxyl and alpha cypermethrin were detected at the same time in one sample S4 taken from the Bugarama valley.

Alphacypermethrin was detected in two samples S3 and S4 from Bugarama market.

The high value of pesticide residues of cyhalothrin is 1.97 ppm was detected in sample collected from Tyazo market and the least detected pesticide residues was alphacypermetrin 0.27 ppm was detected at Bugarama market.

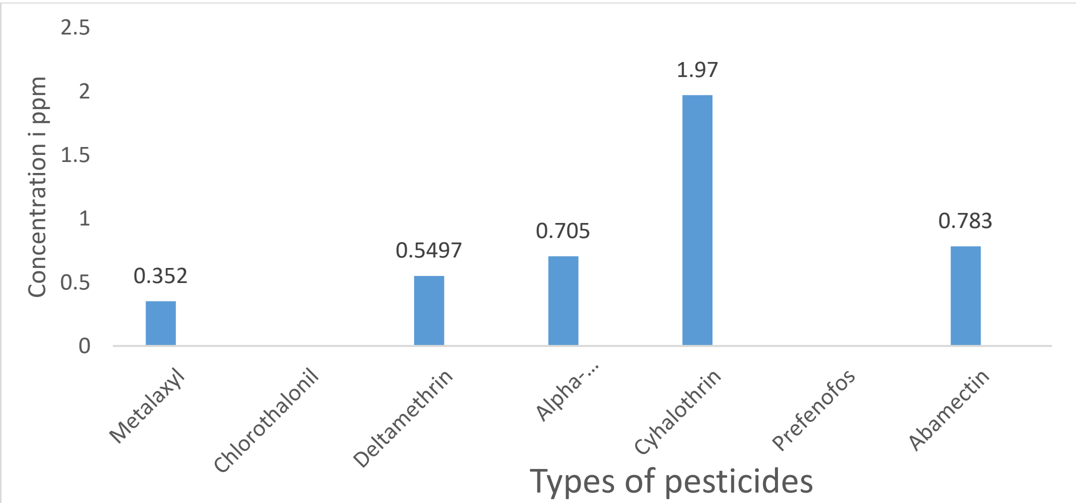

Figure 21: Mean of pesticide residues in eggplant samples.

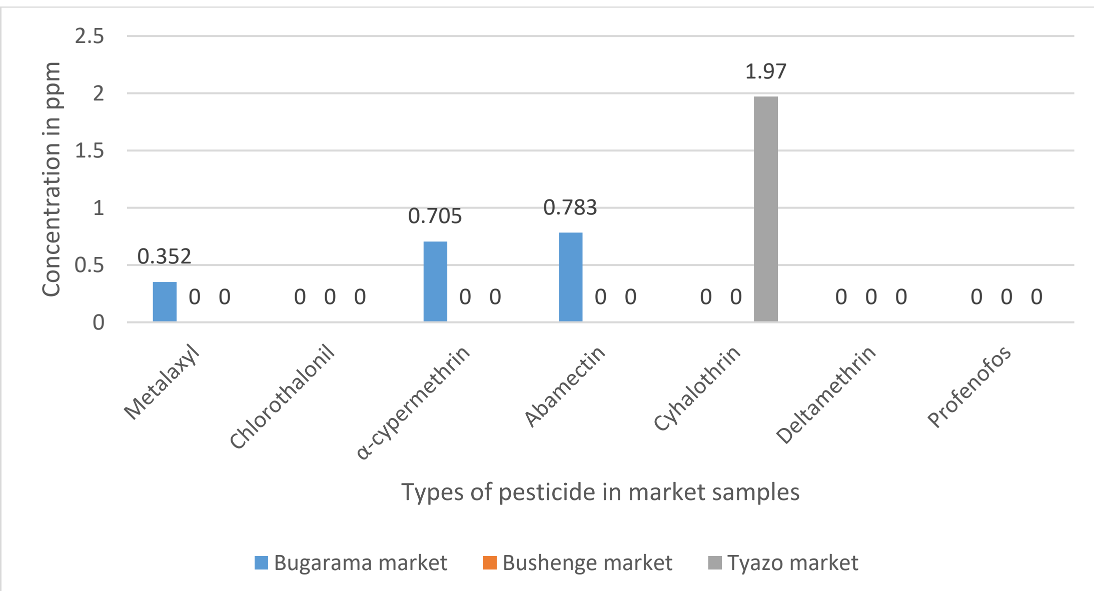

Figure 22 : Mean of pesticide residues in eggplant samples from 3 markets

Analyzed pesticides were detected in 3 vegetable samples at different concentration, eggplant presence the highest pesticides residue 1.97 ppm , followed by tomato (1.789) then cabbage (1.652 ppm) some in the value obtained are less than the maximum residues limits indicated by international food standard regulators (metalaxyl pesticides residues in eggplant 0.352 < 0.5 mg /kg) like, FAO and codex alimentarius [44].

Other values obtained are higher than the maximum residues indicate by these internationals food standard regulators.

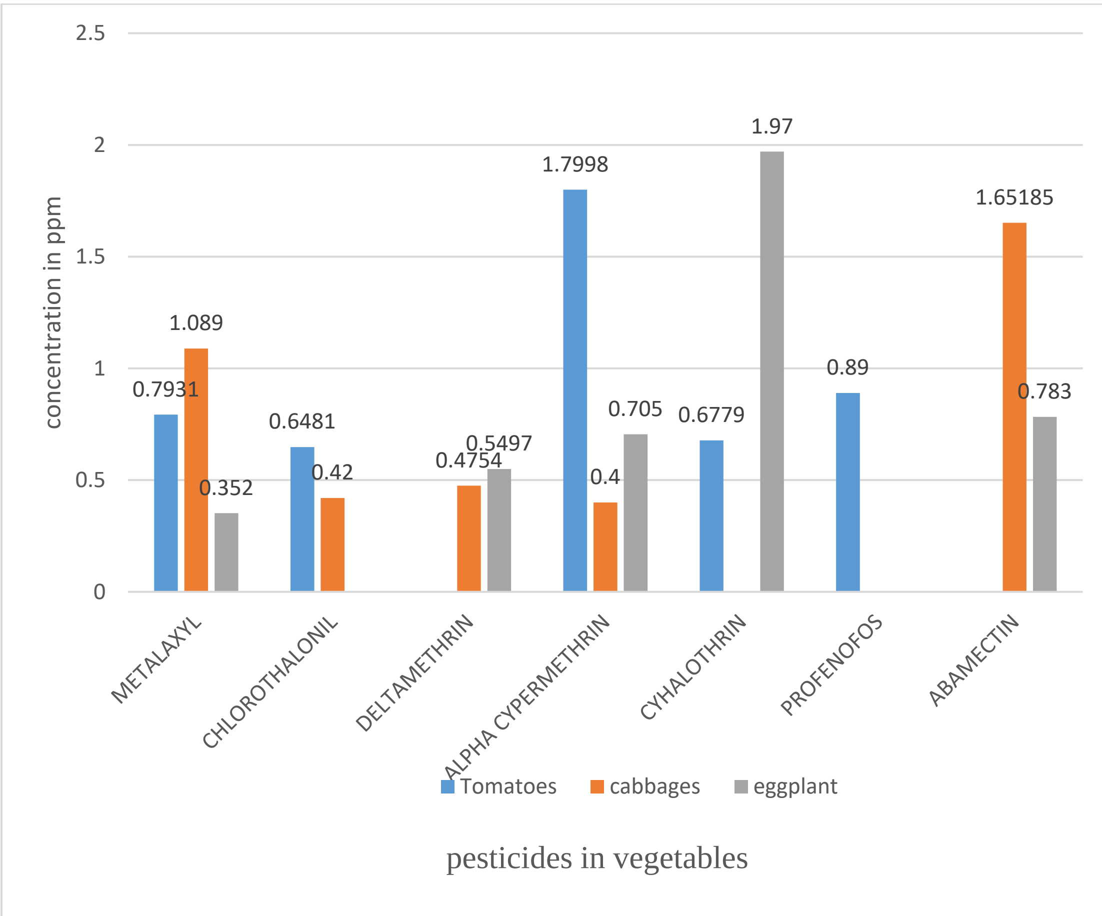

Figure 23: Mean residues of concerned pesticides in analysed vegetables

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

The work focused on assessing the use of pesticides in Rusizi and Nyamasheke districts of western province of Rwanda, the ways in which farmers used pesticides had potential negative impacts on environments. The metalaxyl, abamectin, alpha cypermetrin, profenofos, chlorothalonil and deltamethrine pesticide residues were determined in tomato, cabbage and eggplant. HPLC instrument was used to analyze these 7 pesticdes (metalaxyl, chlorothalonil, alphacypermethrin, Cyhalothrin, Deltamethrin, profenofos and Abamectin) using QuEChERs methods and all of these pesticide residues were detected the HPLC showed a good precision, linearity, limit of detection and limit of quantification.

Some pesticide residues obtained in analyzed vegetable are lower than the standard residues recommended by the international food regulators.

Other pesticide residues obtained in analyzed tomato, cabbage and eggplant are higher than the standard residues recommended by WHO, EU (Appendix. XI) [45]. As the same results were obtained in previous studies this prove the efficacity of the method used in this study [46, 47].

In some cases, the results showed that more than one pesticide residues were present in one sample.

The results obtained prove that once the pesticide are well used by the farmers can improve the crops production and pest managements as the residues levels can be not be detected or be detected at low value recommended by international food regulators .

All seven pesticides were detected on 3 markets samples but the sample from

Bugarama valley are more contaminated compared to other market samples this is probably related to the presence of higher water availability, warmest area so there is a huge amount of pests so farmers tried to use excess pesticides and higher frequency of spraying and do not respect mixing labels for pest control leading to possible intoxication.

The management of the generated wastes during spraying these chemicals, the lack of skills in pesticides application and lack of personal protecting equipment for farmers and pesticides dealers were found also as the major sources of intoxication and environmental pollution.

HPLC instrument was used to analyze these 7 pesticdes (metalaxyl, chlorothalonil, alphacypermethrin, cyhalothrin, deltamethrin, profenofos and Abamectin) using QuEChERs methods and all of these pesticide residues were detected the HPLC showed a good precision, linearity, limit of detection and limit of quantification. The residue pesticide residues obtained some are higher than the standard residues recommended by WHO, EU and other are lower (Appendix. XI) [44], [45].

5.2. Recommendations

More than 70% Rwandan population are occupied by farming so the increasing farmers awareness on pesticide application, training on pesticides uses, protective equipment, handling and spraying materials should be provided to farmers for their safe management.

The researcher should collaborate with farmers and policymakers to improve farmers knowledge and skills on appropriate use of pesticide. The researchers should collaborate with policy markers to develop and integrate a best methods of pest management

A future study on assessment of different pesticide residues in many crops is required. Pesticides pollution control on surface water and soil are also required. The concerned board like RSB, Rwanda FDA, should plan the way out of these highly toxic pesticides, and strengthening the ways of pesticides monitoring and management.

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APPENDIX. I: Questionnaire used during farmers survey

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

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

vegetable………………………….

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. Have you received any training on pesticide management? a) Yes b) self-trained c) no training

5. Frequency of spraying per crop a.< 2 b. 2-4 c. 5-6 d. > 7 7.

6.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. 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. How do you dilute your pesticides? Is it the same as the indication on label? A. it's the same B. not the same

15. 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 directi on A) yes B) No

16. Empty pesticides containers are; A) re-used for domestic purposes B) incinerated C) returned to the dealers D) dumped in the environment.

17. How do you manage the expired pesticides? A) re-used B) stored in good conditions for incineration C) dumped in the environment.

18. 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

19. 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

Appendix IV: Calibration Curves for different pesticides

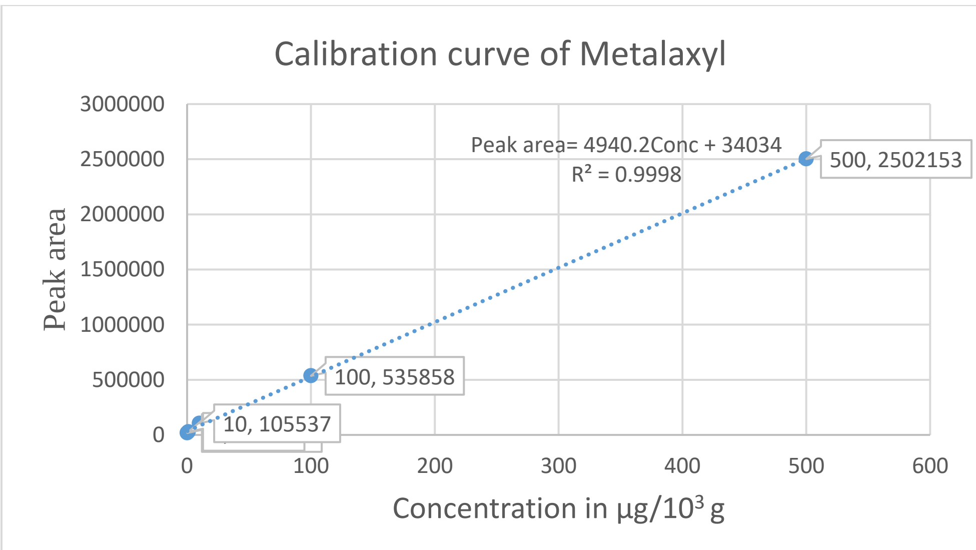

Figure 24: Calibration curve for metalaxyl pesticide

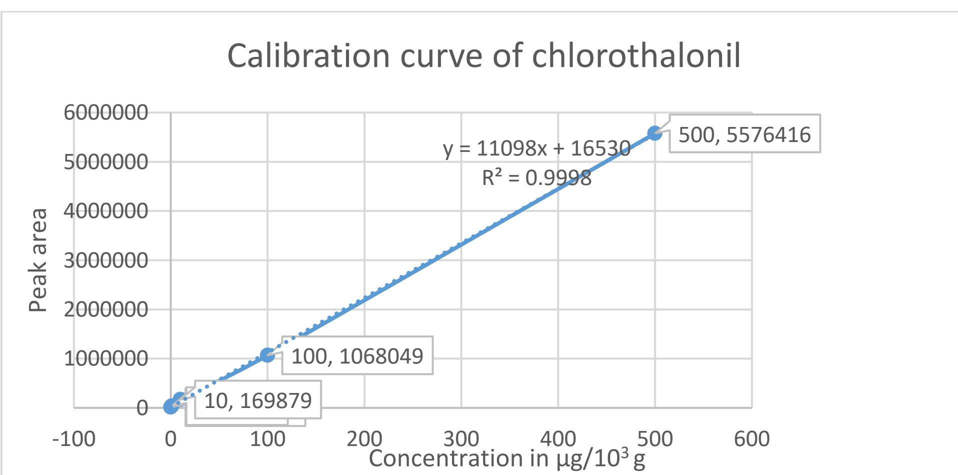

Figure 25 : Calibration of chlorothalonil pesticide

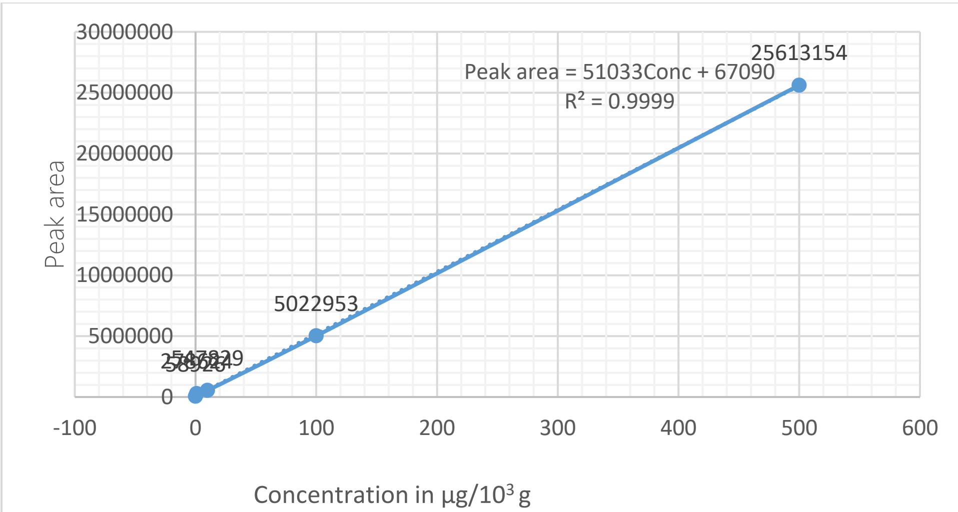

Figure 26: Calibration of abamectin pesticide

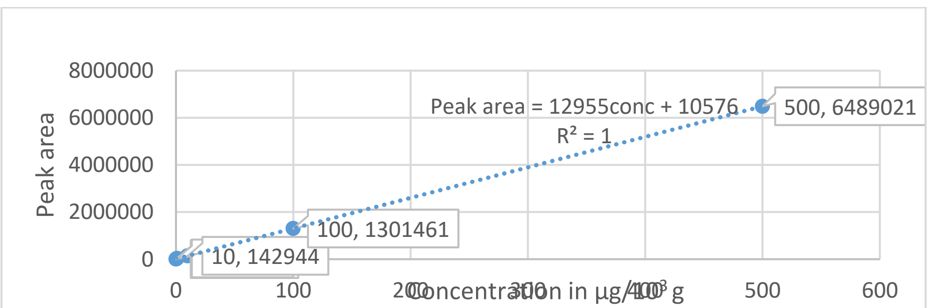

Figure 27: Calibration curve of deltamethrin pesticide

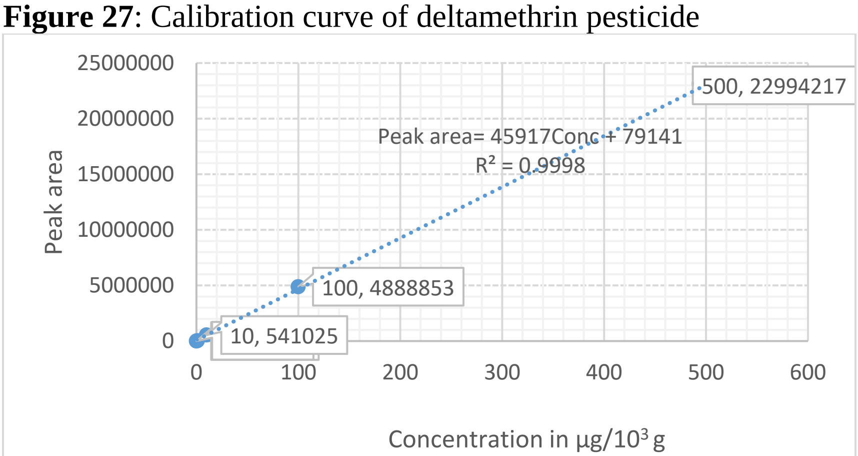

Figure 28:Calibration curve of cyhalothrin pesticide

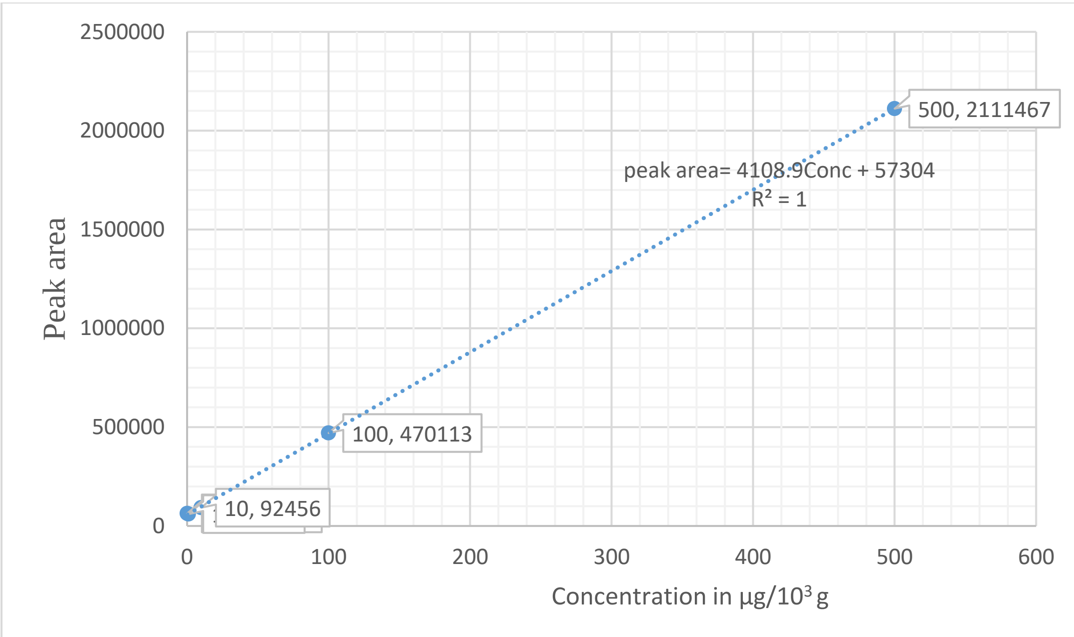

Figure 29: Calibration curve of alpha cypermethrin

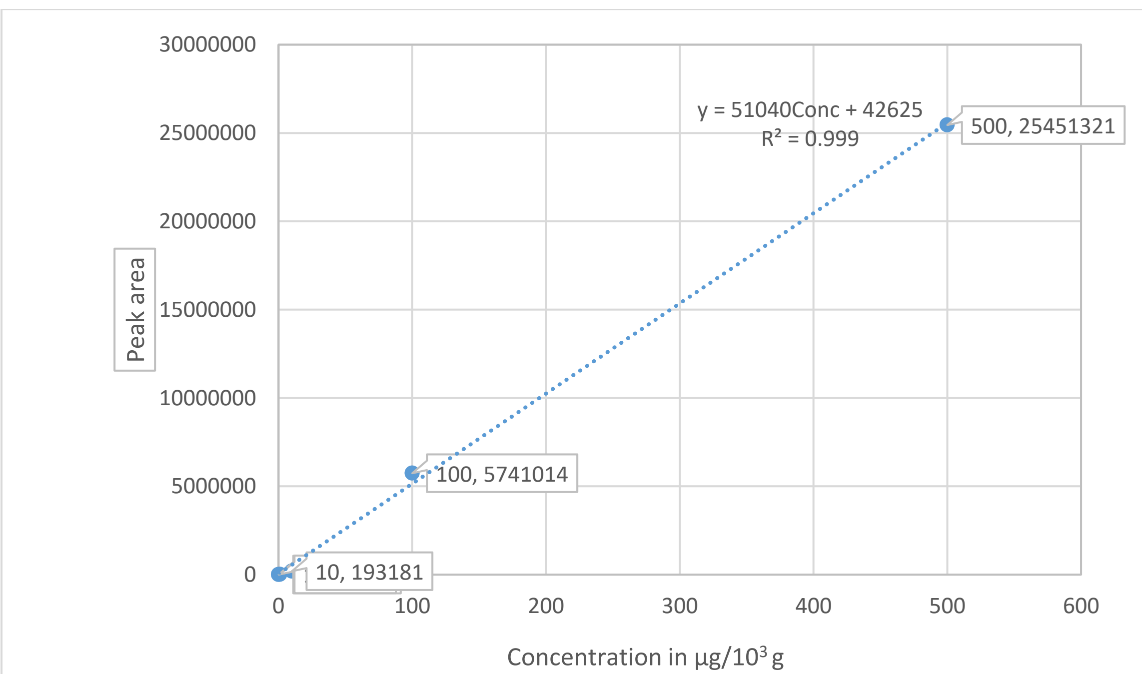

Figure 30: Calibration curve of Profenofos.

Appendix V . Table 2: Limit of detection and limit of quantification and linear equation of analyzed pesticides

Pesticides	Lineaquation	R^2	LOD=3.3*SD/SLOPE	LOQ=3.3*LOD
Metalaxyl	y=49.402x+0.3403	0.9998	0.026720715ppb	0.088178358ppb
Chlorothalonil	y = 110.98x + 0.1653	0.9998	0.028181188 ppb	0.09299792 ppb
Deltamethrin	y=129.27x+ 0.2239	1	0.009431636	0.031124399
Cyhalothrin	y = 459.17x + 0.7914	0.9998	0.02372375	0.078288374
α-cyperimethrin	y = 41.089x + 0.573	$R^2 = 1$	0.00473	0.00893049
Abamectin	y = 510.33x + 0.6709	0.9999	0.018559183	0.061245306
Profenos	y = 510.4x + 0.4263	0.999	0.059222804	0.195435253

Appendix VI. Table 3: Percentage of recovery of analyzed pesticides.

Pesticides	%of recovery in Tomato	% of recovery in cabbage	% of recovery in Egg pants
Metalaxyl	84.5+0.05	86 ±0.05	87.39±0.35
Chlorothalonil	77.83±0.06	78.25±0.05	82.45±0.05
α-cypermethrin	79.15±0.05	117.65±0.05	89.16±0.05
Abamectin	76.53±0.05	70.5±0.05	74.23 ±0.05
Cyhalothrin	88.26±0.043	88.45±0.05	91.34 ±0.05
Deltamethrin	77.12±0.05	74.82±0.05	79.67±0.05
Profenofos	79.14±0.05	76.13±0.05	78.32 ± 0.34

Appendix VII. Table 4 :Table of Results of pesticide residues in tomato

Standard	Metalaxyl	Chlorothalonil	Deltamethrin	αCypermethrin	Cyhalothrin	Profenofos	Abamectin
RT	0.746	1.278	12.751	1.32	1.076	5.72	3.47
Bugarama T S1	-	-	-	-	-	-	-
Bugarama T S2	1.65	0.29	-	-	-	-	-
Bugarama T S3	1.947	1.0062	-	-	-	-	-
Bugarama T S4	-	-	-	-	-	-	-
Bugarama T S5	-	-	-	-	-	-	-
Bugarama T S6	-	-	-	1.7998	-	-	-
Bugarama T S7	1.67	-	-	-	-	-	-
Bugarama T S8	-	-	-	-	-	-	-
Bugarama T S9	-	-	-	-	-	-	-
Bugarama T10	1.9057	-	-	-	-	0.89	-
BushTomato S11	-	-	-	-	-	-	-
BushTomato S12	-	-	-	-	-	-	-
BushTomato S13	-	-	-	-	-	-	-
BushTomato S14	-	-	-	-	-	-	-
BushTomato S15	-	-	-	-	-	-	-
Bush Tomato S16	-	-	-	-	-	-	-
Bush Tomato S17	-	-	-	-	-	-	-
Bush Tomato S18	-	-	-	-	0.6779	-	-
Bush Tomato S19	-	-	-	-	-	-	-
Bush Tomato S20	-	-	-	-	-	-	-
Tyazo Tom S21	-	-	-	-	-	-	-
Tyazo Tomato S22	-	-	-	-	-	-	-
Tyazo Tomato S23	-	-	-	-	-	-	-
Tyazo Tomato S24	-	-	-	-	-	-	-
Tyazo T S25	-	-	-	-	-	-	-
Tyazo T S26	-	-	-	-	-	-	-
Tyazo T S27	-	-	-	-	-	-	-
Tyao Tomato S28	-	-	-	-	-	-	-
Tyazo Tomato S29	-	-	-	-	-	-	-
TyazoTomato S30	-	-	-	-	-	-	-
Mean ± standard deviation	1.793175 ± 0.13416	0.6481±0.3581		1.7998 ± 0.05	0.6779± 0.05	0.89± 0.05	

-: Below detection limit

Appendix VIII. Table 5:Table of results of pesticide residues in cabbage

Standards	**Metalaxyl**	**Chlorothalonil**	**Deltamethrin**	**Alpha Cypermethrin**	**Cyhalothrin**	**Preofenofos**	**Abamectin**
RT	0.746	1.278	12.751	1.32	1.076	5.72	3.47
Cab S1	-	-	-	-	-	-	-
Cab S2	-	-	-	-	-	-	-
Cab S3	-	-	-	-	-	-	-
Cab S4	1.48	-	-	-	-	-	1.6837
Cab S5	-	-	-	-	-	-	-
Cab S6	-	-	-	-	-	-	-
Cab S7	-	-	-	-	-	-	-
Cab S8	-	-	-	-	-	-	-
Cab S9	-	-	-	-	-	-	-
Cab S10	-	-	-	0.55	-	-	1.62
Cab S11	1.24	-	-	-	-	-	-
Cab S12	-	-	-	-	-	-	-
Cab S13	-	-	-	-	-	-	-
Cab S14	-	-	-	-	-	-	-
Cab S15	-	-	-	-	-	-	-
Cab S16	-	-	-	0.25	-	-	-
Cab S17	-	-	0.24	-	-	-	-
Cab S18	-	-	-	-	-	-	-
Cab S19	0.547	-	-	-	-	-	-
Cab S20	-	-	-	-	-	-	-
Cab S21	-	-	-	-	-	-	-
Cab S22	-	-	0.7108	-	-	-	-
Cab S23	-	0.42	-	-	-	-	-
Cab S24	-	-	-	-	-	-	-
Cab S25	-	-	-	-	-	-	-
Cab S26	-	-	-	-	-	-	-
Cab S27	-	-	-	-	-	-	-
Cab S28	-	-	-	-	-	-	-
Cab S29	-	-	-	-	-	-	-
Cab S30	-	-	-	-	-	-	-
Mean ± SD	1.089 ± 0.395578	0.42 ± 0.05	0.4754 ±0.2354	0.4 ± 0.15			1.652 ± 0.032

with - : Below detection limit

Appendix IX. Table 6:Table of results of pesticide residues in eggplant

Standard	Metalaxyl	Chlorothalonil	Deltamethrin	αcypermethrin	Cyhalothrin	Prefenofos	Abamectin
Bug. Egpl S1	0.746	1.278	12.751	1.32	1.076	5.72	3.47
Bug. Egpl S2	-	-	-	-	-	-	-
Bug. Egpl S3	-	-	-	0.27	-	-	-
Bug. Egpl S4	0.352	-	-	1 .14	-	-	-
Bug. Egpl S5	-	-	0.5497	-	-	-	-
Bug. Egpl S6	-	-	-	-	-	-	0.783
Bug. Egpl S7	-	-	-	-	-	-	-
Bug. Egpl S8	-	-	-	-	-	-	-
Bug. Egpl S9	-	-	-	-	-	-	-
Bug. Egpl S10	-	-	-	-	-	-	-
Bush.Egpl S11	-	-	-	-	-	-	-
Bush.Egpl S12	-	-	-	-	-	-	-
Bush.Egpl S13	-	-	-	-	-	-	-
Bush.Egpl S14	-	-	-	-	-	-	-
Bush.Egpl S15	-	-	-	-	-	-	-
Bush.Egpl S16	-	-	-	-	-	-	-
Bush.Egpl S17	-	-	-	-	-	-	-
Bush.Egpl S18	-	-	-	-	-	-	-
Bush.Egpl S19	-	-	-	-	-	-	-
Bush.Egpl S20	-	-	-	-	-	-	-
Tyaz.Egpl S21	-	-	-	-	-	-	-
Tyaz.Egpl S22	-	-	-	-	-	-	-
Tyaz Egpl S23	-	-	-	-	-	-	-
Tyaz Egpl S24	-	-	-	-	1.97	-	-
Tyaz Egpl S25	-	-	-	-	-	-	-
Tyaz egpl S26	-	-	-	-	-	-	-
Tyaz egpl S27	-	-	-	-	-	-	-
Tyaz egpl S28	-	-	-	-	-	-	-
Tyaz egpl S29	-	-	-	-	-	-	-
Tyaz egpl S30	-	-	-	-	-	-	-
Mean ± SD	0.352±0.05		0.5497±0.05	0.705±0.435	1.97±0.05		0.783±0.05

Appendix X . Table 7:Average of pesticide residues detected in analyzed vegetables

Pesticides	Tomato	Eggplant	Cabbage
Metalaxyl	0.79315± 0.13416	0.352±0.05	1.089 ± 0.395578
Chlorothalonil	0.6481±0.3581		0.42 ± 0.05
Deltamethrin		0.5497±0.05	0.4754 ±0.2354
Alpha cypermethrin	1.7998± 0.05	0.705±0.435	0.4±0.15
Cyhalothrin	0.6779± 0.05	1.97± 0.05	
Profenofos	0.89± 0.05		
Abamectin		0.783± 0.05	1.65185 ±0.03185

Appendix XI. Table 8:Maximum residue limits of some alayzed pesticides in vegetable

	MRLs/ Adi Mg/Kg Bw /Day (FAO/WHO)			Average of pesticide residues in analyzed vegetable		
Pesticides	Tomato	Eggplant	Cabbage	Tomato	Eggplant	Cabbage
Metalaxyl	0.5	0.5	0.5	0.79315	0.352	1.089
Chlorothalonil	0.5			0.6481		0.42
Deltamethrin	0.3	0.2	0.2		0.4754	0.5497
Alpha-cypermethrin	0.2	0.03	0.03	1.7998	0.705	0.4
Cyhalothrin	0.5	0.2	0.2	0.6779	1.97	
Profenofos	0.03	0.07	0.03	0.89		
Abamectin	0.05	0.05	0.05		0.783	1.65185

Appendix XV. Images of samples in extraction

These are examples of some tomato,egg plant and cabbage used in extraction