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Centre for Food Science and Nutrition

ASSESSMENT OF PESTICIDE RESIDUE LEVEL IN FRUITS AND VEGETABLES FROM MARKETS OF ADDIS ABABA

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Advisors:

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A THESIS SUBMITTED TO ADDIS ABABA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD SCIENCE AND NUTRITION.

Addis Ababa University

Addis Ababa, Ethiopia

June, 2019

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Declaration

I, the undersigned, declare that this thesis is my original work and that all sources of materials used for the thesis have been duly acknowledged.

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Dr. Tarekegn Berhanu	_____	_____

Abstract

Fruits and vegetables are main sources of vitamins and minerals, thus, they are an essential element of a healthy diet. On the other hand, they may be contaminated with toxic substance such as pesticides residues from farm area, heavy metals, etc. Regulation of pesticide maximum residue limits in food commodities was established in many countries. For Ethiopia, this regulation exists by law but is not fully enforced. Therefore, pesticide residues in fruits and vegetables have not been well monitored. This study is the first extensive assessment to determine the levels of 21 pesticides residues in lettuce, tomato, strawberry and apple sold in Addis Ababa markets. Residues of 13 organochlorine pesticides (heptachlor, aldrin, dieldrin, endrin, γ-chlordane, α-endosulfane, endosulfane sulfate, methoxychlor, α-BHC, p,p DDT, p,p DDE, p,p DDD and δ-BHC) ,3 organophosphate pesticides (malathion, chlopyrifos and diazinon) and 5 synthetic pyrethriod pesticides (λ-cyhalotrhin, cyfluthrin cypermethrin, fenvalerate and deltamethrin) were detected using gas chromatography electron capture detector, and confirmed by gas chromatography mass spectrometry. The effect of washing with tap water was also investigated. In addition, KAP (Knowledge, attitude and practice) study was conducted on consumers. The samples were prepared using the multi-residue **Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe (QuEChERS) method. Of the 21 pesticides, 10 pesticides were detected in 87.5 % of fruit and vegetable samples. The residue concentrations range from 0.92 to 27.09 µg/kg, 5.37 to 198.6 µg/kg and 6.61 to 96.74 µg/kg for organochlorine, synthetic pyrethriod and organophosphate pesticides respectively was observed. Lindane (α-BHC and δ-BHC) and fenvalerate were the most frequent organochlorine and synthetic pyrethriod pesticides, respectively. Lettuce was the most frequent contaminated sample. The concentration levels of organochlorine, synthetic pyrethriod and organophosphate pesticides residues in lettuce, apple and strawberry exceeded the maximum residue limits adopted by Codex and/ or European Union. About 20% of the pesticide residues were in this category. Washing lettuce and strawberry under running water significantly reduced ($p < 0.05$) lindane and endosulfan residues by 32% and 7%, respectively. The running water method did not significantly decrease synthetic pyrethriod residues in all samples. Knowledge, attitude and practice study show that consumers had a moderate level of Knowledge, attitude and practice towards pesticide residues. This research suggests that routine monitoring of pesticide residues is necessary to ensure the public health risks associated with eating contaminated fruits and vegetables. Washing fruits and vegetables before consumption is advisable as this helps to reduce at least some of the pesticide residues from entering into our daily intake.

Key words: Assessment, Organochlorine pesticides, Organophosphate pesticides, synthetic pyrethriod pesticdes, fruits and vegetable

Acknowledgments

First and above all, I would like to praise the almighty God. I would never have been able to finish my thesis without the support of many people and organization.

First of all, I would like to express my earnest appreciation to my advisors Dr. Ashagrie Zewdu and Dr. Tarekegn Berhanu for invaluable support, encouragement and reshaping of my research.

I would like to thank Ethiopian conformity assessment enterprise (ECAE), for giving me the chance of scholarship and financial support throughout my stay. I am very grateful to the ministry of agriculture (MoA) and Ethiopian Veterinary Drug and Feed Administration and Control Authority (VDFCA) providing me pesticide standards, allowing me to use their GC-ECD and laboratory for this research work. I would like also to thank their staff members for overall support in any situation and encouragement throughout my study.

Most importantly, I would like to express my love and deepest appreciation to my beloved Wife Meseret Nigat, of course, Word does not express my feeling exactly, but I must convey my deepest affection and appreciation to her. Throughout my academic career, she has always provided her love, support, and encouragement. Thank you so much indeed it is your greatest support, encouragement and scarifies that brought this work to a successful end. I also thank my family who encouraged me and prayed for me throughout the time of my research.

Finally, I would like to thank all my friends and colleagues who appreciated my work and contributed a lot in one way or another, especially Mr. zerihun Abebe, Mrs. Abeba Habtu, Mrs. Israel Habtu and Mrs. Haymanot Deresse helped me when I need them. Again; I would like to express my thankfulness to Mr. Henok Ashagrie.

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List of abbreviations and symbols

ADI: Acceptable Daily Intake

ANOVA: analysis of variance

AOAC: Association of Official Analytical Chemists

ARfD: Acute Reference Dose

BHC: Benzene hexachloride

CAC: Codex Alimentarius Commission

CSA: Central Statistics Authority

DNA: Deoxyribonucleic Acid

d-SPE: Dispersive-solid-phase extraction

EC: European Commission

ECAE: Ethiopian Conformity Assessment Enterprise

ECD: Electron-capture detector

EFSA: European Food Safety Authority

EPA: Environmental Protection Agency

EU: European union

FAO: Food and Agriculture Organization

GC/ECD: Gas chromatography/ Electron-capture detector

GC/MS: Gas chromatography/ Mass spectrometry

GC: Gas chromatography

GCB: graphitized carbon black

HCH: Hexachlorhexane

HPLC: high-performance liquid chromatography

JMPR: Joint FAO/WHO Meeting on Pesticide Residues

KAP: Knowledge, Attitude and Practice

LC: Liquid Chromatography

LOD: Limit of Detection

LOQ: Limit of Quantification

MgSO4: Magnesium sulfate

MRL: Maximum Residue Limit

OCP: Organochlorine Pesticide

OPP: Organophosphate pesticide

p,p DDD: para para dichlorodiphenyldichloroethane

p,p DDE: para para dichlorodiphenyldichloroethylene

p,p DDT: para para dichlorodiphenyltrichloroethane

POP: Persistent organic pollutants

PSA: primary secondary amine

QuEChERS: Quick, Easy, Cheap, Effective, Rugged and Safe

RSD: Relative Standard Deviation

RT: retention time

SD: Standard Deviation

SPP: Synthetic pyrethroids pesticide

USDA: United States Department of Agriculture

WHO: World Health Organization

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Chapter one

1 Introduction

1.1 Background of the study

Fruits and vegetables play a number of important roles in human health. They are important sources of macro- and micro-nutrients and phytochemicals necessary for boosting body immunity thus maintaining health and preventing diseases in human (Lewis & Ruud, 2004; Sharma et al., 2010). It is in this context that nutrition guidelines contain a recommendation that a balanced diet should include fruits and vegetables. Dieticians prescribe higher amounts of vegetable and fruits consumption for people suffering from non-communicable diseases. As a result, there is an increase in awareness about the health benefits of fruits and vegetables in human diets (WHO, 2003a). Like other crops, fruits and vegetables are attacked by pests and diseases during cultivation through to storage. Pesticides are used to prevent fruit and vegetable from damage, to preserve their quality, to destroy pests and prevent diseases. The use of pesticides however, often leads to the presence of residues in the fruits and vegetables after harvest (Wang et al., 2010).

Pesticide residue refers to pesticides that may remain on or in food after they are applied to food crops. The presence of pesticide residues is a concern for consumers because pesticides are known to have potential harmful effects to other non-target organisms than weeds, pests and diseases. The major concerns are their toxic effects such as interfering with the reproductive systems and fetal development as well as their capacity to cause cancer and asthma (Gilden et al., 2010). These toxic effects are more apparent in fruits and vegetables since they are sometimes consumed without any further processing.

Pesticides used in agriculture production may be categorized into four broad groups; organchlorines (OC), organophosphates (OP), carbamates and synthetic Pyrethroids (SP). Organophosphates and pyrethroids are commonly used pesticides on fruits and vegetables. Organochlorine pesticides (OCPs) were widely used during the last century for agricultural and non-agricultural purposes throughout the world. Even though they have been banned in most countries, due to their high persistence in the environment, their residues are still found in many matrices (Rodrigues et al., 2007). Some pesticides in the OC group that are either banned or

restricted are still in use among Ethiopian farmers (Negatu et al., 2016). Because of this, organophosphates that readily degrade in the environment gained preference over OC. However, OP were found to be highly toxic to mammals and most common cases of acute poisoning occur due to consumption of food contaminated with organophosphates (PAN, 2012). SP have greater photo stability, enhanced insecticidal activity, and relatively low toxicity as compared with organochlorine and organophosphorus pesticides (Zawiyah et al., 2007).

Food safety is a worldwide growing concern on account of its direct relation to human health where the major threats are arising from a wide application of harmful pesticides to control different pests. To protect consumer health and to guarantee that food is safe, the controlling of residues of pesticide in food products must be pursued (Yang et al., 2013). Therefore, the allowed levels of pesticide residues in foodstuffs are legislatively controlled through setting maximum residue levels (MRLs). These MRLs limit the types and amount of pesticides that can be legally present in foods, as determined by various regulatory bodies which minimize consumer exposure to harmful or unnecessary intake of pesticides (Kmellár et al., 2010).

Consumers have to know the different possibilities and how to reduce pesticide intake together with their food. The household, as well as industrial food processing, can influence the level of the pesticide present in the raw agricultural commodity after it is harvested (Amir et al., 2019). Literature revealed that food processing such as washing cooking, peeling, juicing and others may reduce pesticide residue from raw food crops (Fang et al., 2015; Keikotlhaile et al., 2010). Therefore, it is important to investigate the pesticide residues at the point of consumption, mainly after food processing to estimate consumer exposure to pesticides (Mekonen et al., 2019).

Few studies have investigated organochlorine pesticides residues in different food items including, cereals, water, breast milk, honey and in khat (Daba et al., 2011; Gebremichael et al., 2013; Ligani & Hussen, 2014; Mekonen et al., 2014; Mulugeta & Tadese, 2017; Westbom et al., 2008) in Ethiopia and there are no studies regarding OC,OP and SP in fruits and vegetables. The study of the levels of Pesticide residue in food items is very limited in the country. Therefore, the contamination status of fruit and vegetables by pesticide residues at all is unknown. This calls for an extensive study of the residue status of agricultural products.

According to the World Health Organization (WHO), food consumption consists on average of 30% of fruits and vegetables (WHO, 2003b) and it is well known that fruits and vegetables are more contaminated by pesticides than products of animal origin (Claeys et al., 2011). In addition, fruits and vegetables are frequently consumed raw or semi-processed. Moreover, the ways producers of fruit and vegetable spray pesticides and fungicides is tradition and have direct contact with the edible part. As a result, it is expected that they contain higher pesticide-residue levels than other food groups of plant origin such as cereals. This study was aimed to examine the occurrences of OC, OP and SP pesticides residues in lettuce, tomato, apple and strawberry from Addis Ababa markets, and to assess the effect of washing with tap water on the level of residues. In addition, Knowledge, attitude and practice (KAP) study was also conducted on consumers towards pesticide residues.

1.2 Statement of the problem

The increasing demand of fruits and vegetables for local use as well as for export has encouraged the use of pesticide in farming for the purpose of controlling and reducing the effect of insects in food production. Current developmental activities in Ethiopia are resulting in intensified agricultural activity both at small-scale and commercial levels. It is believed that both the types and amounts of pesticides used in Ethiopia are increasing at an alarming rate (Mengistie et al., 2017). The misuse of pesticides was a common problem mainly because farmers lacked appropriate knowledge about pesticides and there was no effective administrative measure governing their use. Moreover, Mekonen et al. (2014) have demonstrated that intensive and improper pesticide use in the field results in pesticide residues that are too high according to the Codex Alimentarius on marketed maize, teff, red pepper and coffee. Some banned pesticides and those not authorized for use in cereals, vegetables, and coffee, such as organochlorines (e.g., DDT and endosulfan), were also detected. Therefore, it is imperative to assess the level of pesticide residues in fruits and vegetables.

1.3 Significance of study

The study focused on presence of organochlorines, organophosphates and synthetic pyrethroids residues in fruits and vegetable. The results of the study will be beneficial to the following:

- The consumers will have awareness either fruits and vegetable sold at Addis Ababa markets contaminated with pesticide residue or not and decide the necessary safety measures to be taken.
- The result of the study will help the regulatory bodies and other stakeholders to set MRLs and implementing monitoring program.
- The findings of the study will serve as a reference material and a guide for future researchers who wish to conduct the same experimental study or any study related to pesticide.
- The findings will fill the existing gap in availability of documented literatures on pesticide residue in fruits and vegetables.

1.4 Research questions

1. Do commercial fruits and vegetables sold at Addis Ababa markets contain pesticide residues, and at what level?
2. What is the prevalence of organochlorines, organophosphates and synthetic pyrethroids residues and compare with the MRLs?
3. Are consumers' aware of pesticide residues in fruits and vegetables and the impacts of its health risk?
4. Does washing reduce the level of pesticide residue?

1.5 Objective

1.5.1 General objective

The main objective of this study is to determine the presence of organochlorines, organophosphates and synthetic pyrethroids residues in fruits and vegetable collected from Addis Ababa markets.

1.5.2 Specific objectives

- To detect and quantify the levels of pesticide residue on selected fruits and vegetables.
- To investigate consumer knowledge, attitude and practice regarding pesticide residue in fruits and vegetables.
- To determine the effect of washing on the reduction of residue level.
- To compare Pesticide residue levels in fruits and vegetables identified to internationally accepted MRL

Chapter two

2 Literature review

2.1 Pesticides

2.1.1 Definition of pesticides

The united nation Food & Agriculture Organization , has outlined a pesticide as "any substance or mixture of substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood product or animal feedstuffs, or substances which will be administered to animals for the control of insects, arachnids, or other pests in or on their bodies. The term includes substances intended for use as a plant growth regulator, defoliant, desiccant, or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to safeguard the commodity from deterioration throughout storage and transport." Within this comprehensive definition, it is implicit that pesticides are toxic. They are intended to prevent, destroy or control specific plants or animals that threaten crops or other useful resources.

2.1.2 Classification of pesticides

Pesticides are one broad area where so many classes exist depending on what is of interest. For instance, Crinnion (2009) have classified pesticides by their target organisms.

In this regard, insecticides, fungicides, herbicides and rodenticides are groups of pesticides that target insects, fungi, herbs (plants) and rodents respectively. In addition to the classes mentioned above, pesticides can also be classified as organic or inorganic pesticides (Cann, 2005). The inorganic pesticides are made from naturally occurring minerals and have varying modes of action including interfering with the conversion of energy within cells which can cause death and desiccation. Some examples of inorganic pesticides are boric acid, silica gel, sodium fluoride and those that contain heavy metals such as mercury, arsenic and lead (Cann, 2005). Organic pesticides consist of compounds containing mainly carbon and hydrogen and may contain other elements such as chlorine, nitrogen, sulfur, tin and phosphorus. Organic pesticides can further be

grouped into synthetic and natural organic pesticides. The synthetic organic pesticide can further be grouped into four major classes as organochlorines, organophosphates, carbamates and synthetic pyrethroids.

Banned pesticides have great effects on the environment, humans and crops are therefore not supposed to be used in Ethiopia. Examples of banned pesticides are DDT, aldrin, dieldrin, heptachlor. Pesticides whose application may lead to unreasonable adverse effects on humans, animals or the environment fall under restricted pesticides. Endosulfan is among restricted pesticides which are currently used in Ethiopia (Mengistie et al., 2017)

Organophosphates pesticides

Synthetic organics containing phosphorus complexes inhibit cholinesterase, an enzyme that regulates the peripheral nervous system; extremely toxic to mammals, birds and fish (generally 10-100 times more poisonous than most chlorinated hydrocarbons) (Daszak et al., 2003). They degrade easily, so their bioaccumulation is rare. Some examples are Chlorpyrifos, malathion and diazinon. There are three main classes of organophosphates and these are type A, type B, type C as summarized below.

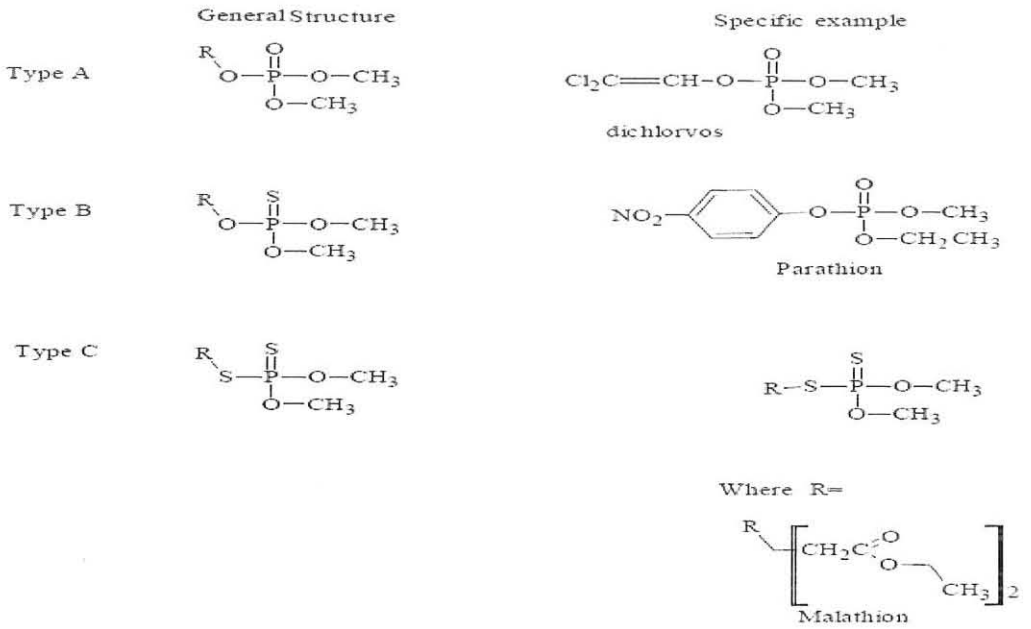


Figure 2. 1 The three main classes of organophosphates with examples.

Organochlorine pesticides

Organochlorine pesticides (OC) are a group of synthetic compounds developed in the 1940s for use mainly as insecticides (Ishaaya et al., 1998). OC belong to the class of persistent organic pollutants (POP), thus they persist in the environment for long periods even after application. Many OC pesticides have been identified as hormone disrupters, exerting their toxic effects on the hormonal and reproductive system thus resulting in adverse health effects to man (Hosie et al., 2000; Waliszewski et al., 2003). Despite the enormous restriction on the use of OCPs due to the entry into force of the Stockholm Convention on POPs (Fiedler, 2007), there is still documented evidence of OC pesticides in food samples (O Akoto et al., 2013; Osei Akoto et al., 2015; Waliszewski et al., 2003) in clouding our country (Daba et al., 2011; Mekonen et al., 2014) . Organochlorine pesticides operate by alteration of ion channels (Abolhassani et al., 2019) thus resulting in neurotoxicity. Chemical structures of some organochlorine pesticides are shown below.

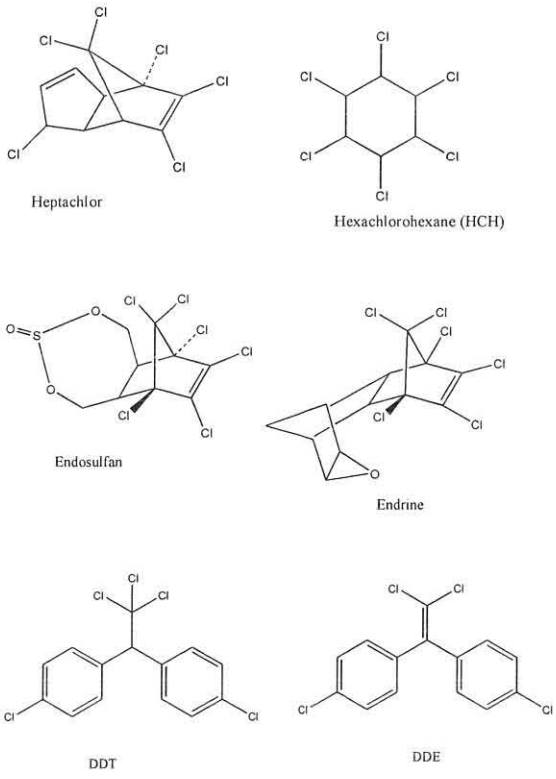


Figure 2. 2 Chemical structure of some organochlorine pesticides.

Synthetic pyrethroids pesticides

Pyrethroids constitute the fourth major group of insecticides developed after OC, OP and carbamate pesticides. Synthetic pyrethroids (SP) are a group of pesticides widely used in agriculture and public health programs worldwide (Heudorf & Angerer, 2001; Lothrop et al., 2007).They are non-systemic, contact and stomach poisons to many insects and arachnids (López-López et al., 2001). The SP mimics the insecticidal function of natural pyrethrins, which are extracts from the chrysanthemums plant. For purposes of insect control in agricultural production, the pyrethroids are preferred over their natural pyrethrin counterparts due to their resistance to environmental degradation, selective insecticidal properties and relative potency (López-López et al., 2001; Saillenfait et al., 2015). The pyrethroids differ from OP pesticides in terms of their lack of a common mechanism of action. Structural examples of some synthetic pyrethroids are shown below.

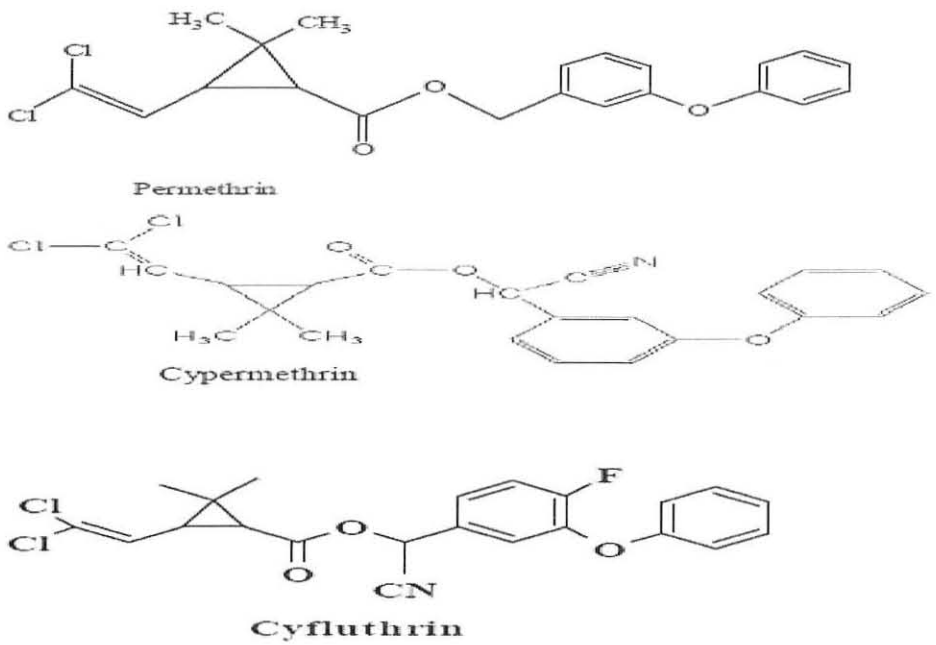


Figure 2. 3 Chemical structure of some synthetic pyrethroids pesticides.

2.2 Common pesticide used in fruits and vegetables

It's nearly impossible to list the pesticides found in all types of vegetables and fruits. There are over 1000 different pesticides that are used to grow crops. Plus there are many pesticides registered for use on the same crop. They include combinations of insecticides, fungicides and herbicides. Also, the specific pesticides used can vary with different growing seasons, different pest problems and the region or country where the crop was grown. Sometimes pesticides end up on crops unintentionally when it drifts from other fields. Plants can also absorb pesticides that linger in soil for years after application. In Ethiopia organophosphates and synthetic pyrethroids are commonly used pesticides on fruits and vegetables production (Haylamicheal & Dalvie, 2009).

2.3 Pesticide residues in fruit and vegetable

Pesticides used on fruits and vegetables have been reported to leave pesticide residues on the exposed crops (Nishina et al., 2010; Szpyrka et al., 2015). These pesticide residues that remain on crops expose human beings to adverse health effects. Because fruits and vegetables are mainly eaten unprocessed, they are the main source of pesticide residue intake for humans (Juraske et al., 2009; PAN, 2012). In a study done by Juraske et al. (2008) it was reported that the intake of toxic substances due to pesticide residues in food commodities could be higher than the intake of these substances related to water consumption and inhalation. Studies have however, shown that some foods may contain pesticide residues above MRLs (Szpyrka et al., 2015; Waliszewski et al., 2003). In a study done in Germany, 2% of all agriculture products of plant origin examined including lettuce for example showed signs of inadmissible application of pesticides. In the same study, the levels of contamination detected in 11 samples of pineapple, tomatoes, peaches, nectarines, lettuce and zucchini were considered significantly high to possibly pose acute health effects (PAN, 2012).

In another study done in Seoul, Korea (Park et al., 2016), pesticide residues were detected in 11 different samples of the selected agricultural products of which 2 samples exceeded the Korea maximum residual limits. In the same study, six out of the nine analyzed dried pepper leaves had pesticide residues and one sample exceeded the Korea MRLs. Though the study concluded that the detected pesticide residues could not be considered a serious public health problem,

continuous investigations and monitoring was recommended. In Poland, (Szpyrka et al., 2015), 376 of 1026 analyzed samples (36.6%) had pesticide residues. In 18 of these samples (1.8%), residues exceeded maximum residue limits. In the same study, substances not recommended for a given crop were detected in 28 (2.7%) of the samples.

A similar study in Turkey that investigated pesticide residues in 1423 samples of fruits and vegetables revealed that a total of 754 samples contained detectable residues at or above Turkish MRLs. Eighty four (8.4%) of the fruit samples and 83(9.8%) of the vegetable samples contained pesticide residues above MRLs (Bakırcı et al., 2014).
Meanwhile, most developing countries do not have consistent programs for monitoring fruits and vegetables for pesticide residues (Kolani et al., 2016). Ethiopia doesn't have any systematic residue monitoring program at all.

2.4 Maximum residue limit (MRL)

Maximum residue levels are the highest levels of residues expected to be in the food when the pesticide is used according to authorized agricultural practices (EFSA, 2010).The MRLs are always set far below levels considered to be safe for humans. MRLs are subject to legal requirements in most of the countries. International harmonization of MRL does not exist at a global level. Even though the Codex Alimentarius provides MRLs for most of pesticides, they are not statutory. National authorities hold the sovereignty in fixing these limits. Therefore these legal limits can widely vary across countries. Regarding pesticide residues, there are as many regulations as countries. The number of pesticides registered and the MRL set, greatly vary from one country to another. Some countries have adopted very severe rules with MRL well below the Codex settings and zero-tolerance provisions for disallowed or prohibited substances or for which a MRL cannot be established due to the lack of toxicological data (Drogué & DeMaria, 2012). In developed regions like Europe the responsibility of the legislation is led by the European Commission (EC) with input from the member states, European food safety agency and the standing committee on the Food Chain and Animal Health (Ambrus & Yang, 2015). In the US, the leading agency is Environmental Protection Agency (EPA) with input from the United States Department of Agriculture (USDA) and the Scientific Advisory Panel. MRL setting can be the responsibility of one or more authorities in a country and normally involves the health, agriculture and environmental agencies (Tritscher et al., 2013).

MRL enforcement can be a responsibility of one or more agencies and may also depend on different food types. MRL setting is based on the national registered good agriculture practice (GAP) data combined with the estimated likely residue from the supervised trials mean residue (STMR), ADI and ARfD (EFSA, 2010). The information is then evaluated by the risk assessment agency like EFSA in EU or Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for codex Alimentarius. Where national or regional MRLs are not available, internationally recognized bodies such as the United Nations Codex Alimentarius Commission MRLs can be used as guidance. MRLs are generally published in open literature or websites of the regulatory bodies for public usage. MRLs may be exceeded because of pesticide misuse, false positives due to naturally occurring substances, differences in national MRLs, lack of registered pesticides and incorrect pesticide application (EFSA, 2010).

Table 2. 1 EU and codex MRLs (mg/kg) of pesticide

Name of pesticides	Class of pesticides	Strawberry (mg/kg)		Tomato mg/kg		Lettuce mg/kg		Apple mg/kg	
		*a	*b	a	b	a	b	a	B
Diazinon	Organophosphate pesticide (OPP)	0.01	0.1	0.01	0.5	0.01	0.5	0.01	0.3
Malathion	Organophosphate pesticide (OPP)	0.02	1	0.02	0.5	0.5	0.5	0.02	0.5
Chlorpyrifos	Organophosphate pesticide (OPP)	0.2	0.3	0.01	NE	0.05	NE	0.01	1
Endosulfan	Organochlorine pesticide (OCP)	0.1	NE	0.05	0.5	0.05	NE	0.05	NE
Total DDT	Organochlorine pesticide (OCP)	0.5	NE	0.05	NE	0.05	NE	0.05	NE
Cyfluthrin	Pyrethroid	0.02	NE	0.05	0.2	1	NE	0.2	0.1
Cypermethrin	Pyrethroid	0.07	0.07	0.5	0.2	2	0.01	1	0.7
Fenvalerate	Pyrethroid	0.1	ne	0.1	NE	0.2	NE	0.1	NE
Deltamethrin	Pyrethroid	0.02	0.2	0.07	0.3	0.5	2	0.2	0.2
Lambda cyhalothrin	Pyrethroid	1	0.2	0.1	0.3	0.5	NE	0.08	0.2
Dieldrin	Organochlorine pesticide (OCP)	0.02	0.05	0.01	NE	0.01	NE	0.01	0.05
Heptachlor	Organochlorine pesticide (OCP)	0.1	NE	0.01	NE	0.01	NE	0.01	NE
Methoxychlor	Organochlorine pesticide (OCP)	0.1	NE	0.01	NE	0.01	NE	0.01	NE
Lindane	Organochlorine pesticide (OCP)	0.01	NE	0.01	NE	0.01	NE	0.01	NE
Chloridane	Organochlorine pesticide (OCP)	0.02	0.02	0.01	0.02	0.01	0.02	0.01	0.02
Endrin	Organochlorine pesticide (OCP)	0.1	NE	0.01	NE	0.01	NE	0.01	NE

Source : * a http://ec.europa.eu/food/plant/pesticides/eu-pesticides

b http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/en/

NE= not established

2.5 Public health hazards and harmful effects of pesticide residues

The harmful effects of pesticide usage cannot be ignored despite its numerous benefits. Pesticides can cause injury to human health as well as the environment. Exposure to hazardous pesticides is associated with a wide range of adverse effects in humans. Pesticide residues in food can cause short term or acute effect such as dizziness, diarrhoea and death. They can also result in long term or chronic health effects which can occur months or years after exposure.

Chronic health effects include cancer, tumours, brain and nervous system damage, birth defects, infertility and other reproductive system deformities, body organ damage, mild cognitive dysfunction and damage to the immune system (Darko & Akoto, 2008).

Injuries may be caused either by a single massive dose being absorbed during one pesticide exposure, or from smaller doses absorbed during repeated exposures over an extended period of time. Illness or damage is referred to as acute when it has a sudden onset and lasts for a short period of time. The type and severity of the symptoms depend on the chemical mode of action and toxicity and the amount of chemical the victim has been exposed to. WHO estimates of 2000 showed that each year three million farmers in the developing world were experiencing severe poisoning from pesticides each year, about 18,000 of who would die (WHO, 2010).Fifty percent (50%) of the modern pesticides are mutagens, i.e. cause heritable changes in the genetic material, DNA.

Chronic toxicity refers to the effects that occur after exposure over a long period of time, or to symptoms that occur long after exposure and/or persist for a long time. In general, these effects can occur with doses as low as a few micrograms of pesticide per kilogram body weight of the person or animal exposed. Examples of the chronic effects of pesticides on humans are described below.

Pesticides can exert a carcinogenic effect through a variety of mechanisms, including:

Genotoxicity: Pesticides react with DNA to cause mutations or cancer.

Hormonal action: Pesticide lengthens the estrous cycle, prolonging exposure to endogenous estrogen, and can cause mammary and uterine tumours.

Immunotoxicity: Pesticides can alter immune function in a number of ways that can cause cancer.

According to Fox et al. (2012), there is a growing concern in regard to the developmental neurotoxicity by recent epidemiologic observations that children exposed prenatally or during early postnatal life suffer from various neurological effects. Examples of neurological effects are numbness or weakness of arms, legs, feet or hands; lethargy; memory loss; loss of concentration; and anxiety.

2.6 Process effect on pesticide residues

Fruits and vegetables are generally perishable by nature. This can lead to substantial losses to both farmers and consumers. The main objective of processing fruits and vegetables is to supply wholesome, safe, nutritious and acceptable food to the consumers throughout the year (Fellows, 2009). Food processing refers to those methods and techniques that are applied to raw ingredients to transform them into a consumable form. Food processing also includes basic raw material preparation such as washing, removing contaminants and foreign bodies as well as peeling and trimming (removing non-consumable parts of the raw agricultural commodity). These types of categories of processing will be discussed below with reference to their application in fruits and vegetables. The processing techniques identified were: washing, peeling, juicing and boiling.

Washing

Washing is an important step normally carried out after removing the most spoiled fruits and vegetables from the harvest. Washing removes soil, micro-organisms and chemical residues from the fruits and vegetables. Washing can be done by immersion and spraying or showering. Washing is usually carried out using water or water mixed with some sanitizers like 1.5% hydrochloric acid solution. Sometimes warm water can be used (about 50°C) in the pre-washing phase of industrial operations. However, caution should be exercised in using warm water since it can accelerate chemical and microbial degradation if further processing is delayed (Fellows, 2009). At household level, washing is carried out mostly under running tap water while swirling the strainer or colander for a few seconds (Soliman, 1999).

Peeling

Peeling removes those unwanted or indigestible parts of fruits and vegetables mainly the skin or outer leaves. It can be done mechanically by knife or abrasion and flash steaming or chemically by caustic soda at high temperatures (90 – 100°C). In home processing the common method is knife peeling or using other hand held devices like potato peelers (Holland et al., 1994).

Juicing

Juicing of fruits and vegetables after cleaning steps is done by crushing, grinding or disintegration depending on the type of product being processed. In industrial production, the above steps are usually followed by enzyme treatment, heating, pressing, juice clarification and filtration. In home processing the crushing steps are followed by pressing to extract the juice. With the availability of home processing units, these steps can be combined and the juice be obtained immediately (Keikotlhaile et al., 2010).

Boiling

The effects of boiling were said to depend on the time, temperature, degree of moisture loss and whether the system is close. The rates of degradation and volatilization of residue were said to be increased by the heat involved in cooking. Losses of heat stable compounds were expected to be low or increase because of moisture loss during cooking. Boiling of fruits and vegetables in studies reviewed by Holland et al. (1994) showed a reduction of pesticides ranging from 0 to 100%.

2.7 Pesticide residue analysis

More than 800 different kinds of pesticides are used for the control of insects, rodents, fungi and unwanted plants in the process of agricultural production. Although most of them leave the products or degrade in soil, water and atmosphere, some trace amounts of pesticide residues can be transferred to humans via the food chain, being potentially harmful to human health (Gilbert-López et al., 2009).

The analysis of pesticide residues in foods consists of sample preparation and the instrumental determination. Although the analytical instruments are developing rapidly (Ridgway et al.,

2007), their detector noise, detection limits, and final quantification are usually influenced by the interferences from food matrices (Ferrer et al., 2005; Hajšlová & Zrostlıkova, 2003) Thus, the sample preparation is the bottleneck for the effective and accurate analysis of trace pesticide residues (Zöllner et al., 2003). The aim of the sample preparation is to isolate the trace amounts of analytes from a large quantity of complex matrices and eliminate the interferences from the food matrix as much as possible. Typical sample preparation steps include the sampling/homogenization, extraction, and clean-up. Among them, the extraction and clean-up steps play a critical role in the success of pesticide residue analysis.

The traditional sample extraction methods, especially liquid-liquid extraction, have been widely used for pesticide residue analysis. However, these methods are laborious, time- and solvent-consuming, and subject to the loss of analytes due to the tedious experimental procedure. Therefore, new extraction and clean-up methods have been introduced in the field of pesticide residue analysis in foods. These include: supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, gel permeation chromatography, solid-phase extraction, QuEChERS and others (Zhang et al., 2012).

Analytical methods are needed to screen, quantify and confirm the pesticide residues in fruits and vegetables for both research and regulatory purpose. Multiresidue methods (MRMs) and single residue methods (SRMs) generally consist of the same basic steps, but MRMs are preferred to the latter for the analysis of pesticides, because MRMs provide the capability of determining different pesticide residues in a single analysis run.

The method adopted by the Association of Official Analytical Chemists (AOAC) is the internationally recognized procedure for MRM. It involves certain basic steps in the application which includes sample preparation, extraction cleanup and finally the analyte determination is performed by gas chromatography (GC) or high-performance liquid chromatography (HPLC) with selective detectors (Torres et al., 1996).

Several multiresidue methods employing gas chromatography electron capture detector (GC-ECD) to determine pesticides in fruits and vegetable have been evaluated by various workers (Biswas et al., 2019; Farina et al., 2017; Paz et al., 2017; Tankiewicz, 2019). The success of the ECD prompted the development and application of other selective detection principles for non-

halogenated pesticides. GC is the most widely used technique in pesticide analysis because of its high selectivity and resolution, wide dynamic concentration range, good accuracy and precision. At present, more than 60% of registered pesticides and/or their metabolites are amenable to GC (Mach & Verbeck, 2018)

Electron Capture Detectors (ECDs) are often used in pesticide residue determination. It has a very high sensitivity to polychlorinated hydrocarbons and other halogenated pesticides. When electronegative compounds enter the ECD cell from the column, they immediately combine with some of the free electrons, temporarily reducing the number remaining in the electron cloud. When the electron population is decreased, the pulse rate is increased to maintain a constant current equal to the standing current. The pulse rate is converted to an analog output, which is acquired by the Peak Simple data system. Unlike other detectors which measure an increase in signal response, the ECD detector electronics measure the pulse rate needed to maintain the standing current (Hong et al., 2017). It's disadvantaged to selectivity because all kinds of electron attracting functional groups such as nitro groups and aromatic structures also give response (Torres et al., 1996).

2.8 Fruit and vegetable in Ethiopia

The climatic and soil conditions of Ethiopia are suitable for the production of a wide range of tropical and subtropical fruits and vegetables including tomato, lettuce, strawberry and apple. According to information obtained from the Central Statistics Authority (CSA), the total area under fruits & vegetables is about 12,576 hectares in 2011. Of the total land area under cultivation in the country during the same year, the area under fruits and vegetables is less than one per cent (i.e. 0.11%), which is insignificant as compared to food crops. On average more than 2,399,566 tons of vegetables and fruits are produced by public and private commercial farms, this is estimated to be less than 2 percent of the total crop production. Including areas under production of fruit and vegetable crops from commercial private farms and the area and production from the private peasant holdings are indicated in the following table. However, the contribution of horticultural crops both to the diet and income of Ethiopians is insignificant. In recent year with the aim of insuring food security the agricultural development of the country has been due attention. Among the sector that has been given attention at micro level is the production of fruit and vegetables (Smith et al., 2005). Fruit and vegetable production in

Ethiopia is scattered throughout the country on patches of land in peasant smallholder farm whereas, large scale production and processing of fruits and vegetables is carried out only by state organizations (Girmalem, 2011) The country has a variety of fruits, leafy vegetables, roots and tubers adaptable to specific locations and altitudes. Shallot, garlic, potatoes and chilies are mainly produced under rain fed conditions.

Tomatoes, carrots, lettuce, beetroot, cabbage, spinach and Swiss chard are usually restricted to areas where irrigation water is available (Bekabil, 2014). According to the CSA of Ethiopia the 2007/2008 annual of fruit and vegetable production by private peasant holding are described in table 2.2 below.

On the other way consumption of fruits and vegetables in Ethiopia is low compared to developed countries (Machado et al., 2006). The main reason for the low consumption of fruit and vegetables is the eating habit of the society.

Table 2. 2 Area, production and yield of Horticulture crops for private peasant holdings (2007/08)

Crop	Area in Hectare	Production in quintal	Yield (quintal/ hectare)
Vegetables	119,091.43	4,719,664.46	
Ethiopian Cabbage	28,470.69	2,383,602.95	83.72
Tomatoes	4,800.07	338,380.91	70.49
Green peppers	7,952.35	623,209.04	78.37
Red pepper	75,340.93	1,223,996.86	16.25
Root crops	184,329.45	15,309,489.12	
Beetroot	1,840.47	169,479.87	92.09
Onion	18,012.55	1,751,061.71	97.21
Potatoes	50,488.00	4,025,080.08	79.72
Sweet potato	62,357.65	5,264,870.43	84.43
Fruit crops	62,731.42	4,621,475.23	
Avocado	6,473.22	428,292.20	66.19
Banana	39,425.90	2,610,592.27	66.22
Orange	3,396.81	428,072.76	126.02
Pineapples	86.74	448.95	5.18

Source; CSA 2011.

2.9 Trends in pesticide use in Ethiopia

Agriculture in Ethiopia forms the basis of the country's economy. About 84% of the country's population are engaged in agriculture and generate income for their households to sustain their livelihood. The government has committed itself to intensifying the sector through technological advancement and the use of state-of-the-art agricultural inputs such as fertilizers and pesticides.

Ethiopia's agriculture used to be mainly dominated by small-scale farmers practicing subsistence farming, which are dominated by low inputs and low technology farming systems. This was considered to be the main cause of the low production and productivity of farmers; hence the government is promoting the use of agrochemicals throughout the country to increase production and productivity (CSA, 2012)

The use of pesticides in Ethiopia to control crop pests can be traced back to the mid-1940s, when arsenic and later on BHC in bran bait were used to control desert locust outbreaks. The use of agricultural inputs including pesticides was introduced to smallholder farmers since the 1960s via agricultural extension systems. Since then the use of pesticides has shown a steady growth and with the current development of the flower-growing sector, average imports of pesticides have grown to over 2400 tons per annum (Teklu, 2016). The import and use of pesticides in Ethiopia has grown rapidly, as this is also part of a development plan to intensify agriculture with the objective of increasing food production and expanding the floriculture industry (Amera & Abate, 2008).

Commercial farmers, as the main users of pesticides, account for the use of about 80% of the pesticides imported into Ethiopia. The remaining 20% of the total import is used for small-scale farming, household, health and industrial purposes (Teklu, 2016). Of the total of 4125 metric tons of active ingredients that were used in Ethiopia in 2010, the largest proportion (75%) were herbicides, followed by insecticides (15%) and fungicides (9%) (B. Negatu, 2017) .There were 82 cut-flower companies covering 3,101 hectares of land by 2013. Most of the cut-flower production is exported to the Netherlands which has 86% of share by quantity. Other market destinations include Saudi Arabia (3.9%) and Norway (2.6%). Most of the fruit and vegetables are exported to Somalia (57.5%) and Djibouti (37.6 %) in 2013 (B. Negatu, 2017).There was an 8-fold increase in export income (29 to 245 million US dollars between 2005 and 2014) from the

horticultural farms, with similar trends in both cut-flower and fruit and vegetables sectors, but a much steeper trend in cut-flower production.

According to Mengistie et al. (2015), there are now 302 commercial pesticides registered and imported in the country, representing over 160 active ingredients, and the volume of imports increases from year to year. Among these, the largest proportion falls under class II of the WHO hazard classification system (Mengistie et al., 2015). In the recent past, the misuse of pesticides was a common problem mainly because farmers lacked appropriate knowledge about pesticides and there was no effective administrative measure governing their use. For instance, DDT, which has been a banned pesticide since the 1970s world-wide, remains in use in Ethiopia for the control of the mosquito malaria vector by the ministry of health and has been reported to have been illegally diverted to agricultural pest control in some areas (Mengistie et al., 2015). In spite of their ban, aldrin and dieldrin have recently been found in the soil in Ethiopia and organophosphate pesticides, such as diazinon and malathion, are still commonly used in agro-industries and frequently enter the food chain (Daba et al., 2011; Westbom et al., 2008). Scenarios for the future use of seven selected pesticides indicated that agricultural use of chlorothalonil, deltamethrin, endosulfan and malathion in some crops may result in medium to high risks to aquatic species in the Ethiopian context (Teklu, 2016). In addition, Negatu et al. (2016) report a large increase in pesticide usage intensity, illegitimate usages of DDT and Endosulfan on food crops and direct import of pesticides without passing through the formal Ethiopian registration process. Gebremichael et al. (2013) report the presence of DDT from their analysis of organochlorine pesticide residues in human and cow's milk in Southwestern Ethiopia. Moreover, Mekonen et al. (2014) report one-third of maize, teff, red pepper and coffee samples were contaminated with pesticide at a level of above MRLs. Various studies in Ethiopia and other parts of the world have focused much on the analysis of only organochlorine pesticides because of their persistence, lipophilicity, toxicity and bioaccumulation. Overall, however, there are only a few data on pesticide residue contamination of food items. So far regular survey studies and monitoring programmes of the other different types of pesticides including organochlorine have not been carried out in Ethiopia.

Chapter three

3 Materials and methods

3.1 Study design

The design of the study was cross-sectional study involving laboratory investigation to determine the level of selected organochlorine, organophosphate and pyrethroids pesticides in selected fruit and vegetable collected from markets in Addis Ababa and a structured questionnaire was prepared to assess consumers' knowledge, attitude and practice related to pesticide residue. In addition washing treatment was performed for samples containing pesticide residue to investigate its effect on the level of pesticide residue.

3.2 Study area

The study was conducted in Addis Ababa, the capital and largest city of Ethiopia. It lies at an elevation of 2,200 meters (7,200 ft.) and located at 9 degrees 1 minutes north and 38 degrees 44 minutes east. As a capital city, Addis Ababa is a major trade center for fruits and vegetables with many varieties. The city has 10 sub cities each having different number of Woredas (Figure 3.1).

The largest and busiest markets in Ethiopia are found in Addis Ababa. These markets are well known for their massive sales of fruits and vegetables that come from different areas of the country where pesticides are widely used. Thus, assessment of pesticides in samples from these markets could reflect the contamination status of fruits and vegetables.

Experiments were carried out from December 2018 to May 2019 in Ethiopian conformity assessment enterprise (ECAE) laboratory and plant health and production quality control pesticide residue testing laboratory of the ministry of Agriculture, Addis Ababa.

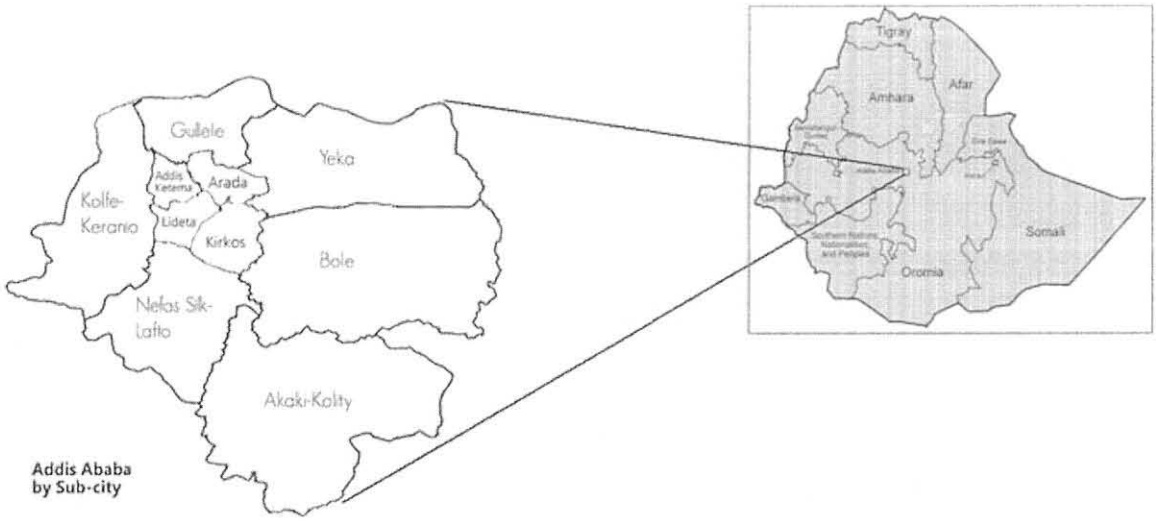


Figure 3. 1 Location of the study area (source:-Addis Ababa city administration website)

3.3 Samples and Sample Preparation

Fruits and vegetables that were analyzed for pesticides residue were apple, strawberry, tomato and lettuce. In order to address this study, cross sectional approach was followed. The cross section analytical study design was appropriate for the quantitative study as it investigated the presence of pesticide residues in fruits and vegetables at a particular point in time. A total of 120 samples were purchased randomly from wholesale distributor, supermarkets, roadside markets, fruit and vegetable stores. From each items, a sample size of at least 1 Kg was purchased. Wholesalers, retailers and consumers are sourcing their fruit and vegetables at these markets. Except for apple samples, the rest were collected from local sources. Apple samples were local and imported sources. After collection, as shown in the figure 3.2, samples were sealed in polyethylene bags, labeled appropriately and transported to Ethiopian conformity assessment enterprise laboratory. All samples were taken and prepared in accordance with Codex Alementaris commission for residual analysis (CAC, 1999). Using composite sampling techniques 120 samples were reduced to 40, and 10 for each samples.

Each sample was divided in to two groups: washed sample and unwashed sample. The edible parts of the samples (washed and unwashed) were homogenized using a blender to obtain thoroughly mixed homogenates. The homogenized samples were placed into amber glass bottles and kept in a deep freezer (-20 ^{o}C) until analyzed. Prior to use, the samples were thawed at 4 ^{o}C overnight.

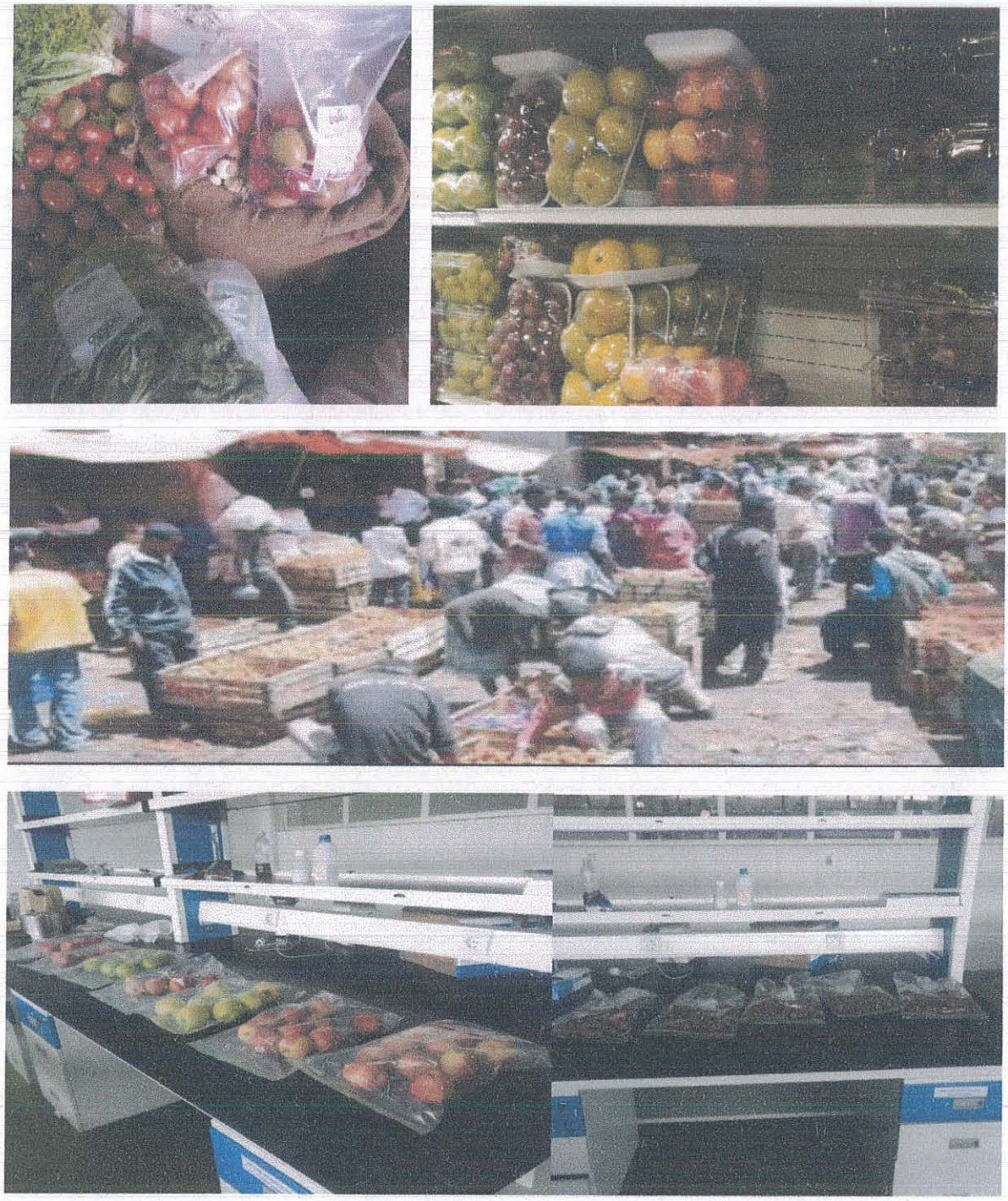


Figure 3. 2 Fruit and vegetable samples and sampling place.

3.4 Chromatographic analysis

3.4.1 Chemicals and reagents

Anhydrous magnesium sulfates ($MgSO_4$), a graphitized carbon black (GCB) sorbent and primary secondary amine (PSA) were purchased from Supelco (Bellefonte, USA). Anhydrous Sodium acetate (NaAC) was obatined from Fisons scientific (Germany). 21 target pesticides were from 3 classes, organochlorine (13), organophosphate (3) and pyrethriod (5), and include α- BHC (99.8%), diazinon (98%), δ- BHC (99.4), heptachlor (99.1%),aldrin (99.5%), chlorpyrifos (97.5%), malathion (98.7%) ,γ-chlordane (99.9%), α-endosulphan (99.6%), p,p DDE (99.8%), dieldrin (97.1%), endrin (99.3%), p,p DDD (99.7%) ,p,p DDT(99.5%) ,endosulphan sulfate (96.6%) ,metoxychlor (98.7%), λ-cyhalotrhin (97.8%), cyfluthrin (98%), cypermethrin (99.7%), fenvalerate (99%) and deltamethrin (99.6%). The 19 certified pesticides standards were obtained from Sigma–Aldrich (St. Louis, MO, USA) and 2 pesticides were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

3.4.2 Preparation of standard solutions

Individual Stock standard solutions were prepared at 1000 mg /kg by dissolving 10 mg of each compound into 10 ml of n-hexane and they were stored at −20 °C. From the individual stock standard solutions, a multi-compound intermediate solution was prepared with a concentration of 10 mg/ kg for OCPs and 50mg/kg for OPPs and synthetic pyrethroid. The intermediate standard solutions that were used for the preparation of matrix-matched standards were within the range of 0.001 to 0.2 mg/kg for OCPs and 0.005 to 1 mg/kg for OPPs and pyrethriods. While not in use, the intermediate standard solutions were kept at −20 °C.

3.4.3 Instruments and apparatus

General instruments

A vortex mixer (Assistant, Germany), electronic analytical balance Mettler-Toledo (PL602-L, Switzerland), nitrogen evaporator (Thermo scientific, USA), homogenizer (Foss Homogenizer, model. 2094, Germany), water purifier (Thermo scientific, USA) and a Sigma centrifuge (4-16K, Germany) were used. Additionally, a freezer, ultra-wave sonicator, pipettes, spatulas, glass

volumetric, oven, muffle furnace, polypropylene centrifuge tubes (15 and 50ml), solvent dispensers, gloves, beakers, glass syringe, filters and vials were used for the extraction protocol.

Gas chromatography instruments and apparatus

The GC analysis was conducted using a GC–microcell electron capture detector (μECD), and the detected pesticides were confirmed by GC–MS as show in figure 3.3 right sides. The GC– μECD analyses were performed on an Agilent 7890 A equipped with a split/splitless injector and a 7693 auto-sampler (Agilent, Santa Clara, USA). Data acquisition and analysis were performed with Chemstation software (Agilent Technologies). The carrier gas and make up gas were nitrogen (99.999%) at a flow rate of 1.0 and 20 ml/min respectively. The separations were performed using Stx-CL pesticide capillary column, 30m long with internal diameter and film thickness of 0.25 mm and 0.20 μm (Agilent, Santa Clara, USA) respectively.

The GC–MS analysis was performed on an Agilent 7890B GC equipped with Turbo MSD 5977A and a 7693 auto injector (Agilent, Santa Clara, USA) as shown in figure 3.3 left sides. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Separations were conducted using a DB-5 30 m x 0.25 mm x 0.25 μm column (Agilent, Santa Clara, USA). A volume of 2 μL of extract was injected in the splitless mode, and the injector temperature was held at 250 °C for the GC–ECD and GC–MS analyses. The same oven temperature was programmed for the GC–ECD and GC–MS as follows: initial temperature of 120 °C (held for 1 min), increased to 225 °C at 14 °C /min (held for 5 min), raised to 280 °C at 6 °C /min (held for 0 min) and, finally, increased to 295 °C at 30 °C /min (held for 10 min). The total chromatography run time was 33 minutes.

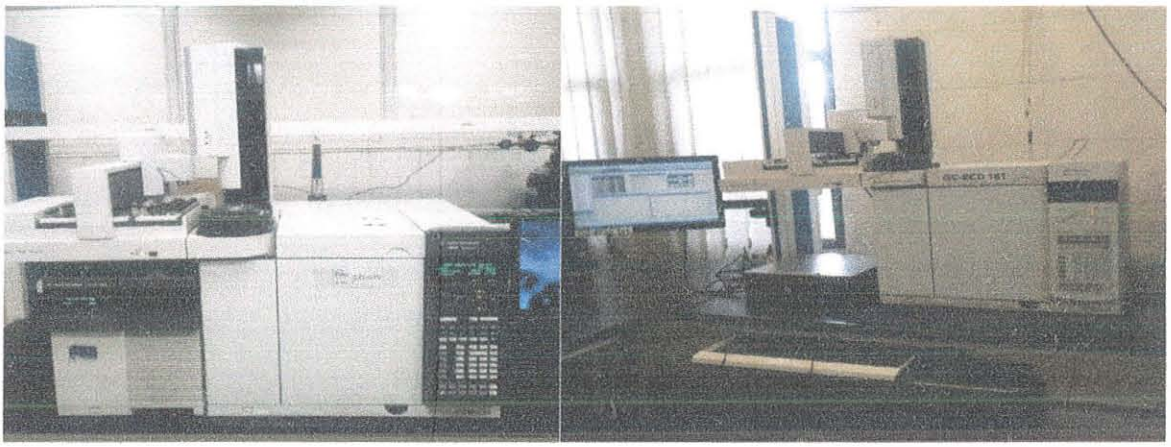


Figure 3. 3 GC/MS and GC/ECD instruments at Ethiopian conformity assessment enterprise and plant health and production quality control pesticide residue testing laboratory, Addis Ababa respectively.

3.4.4 Quality Control and Assurance

Quality control and quality assurance were incorporated into the analytical scheme. Samples were carefully handled to avoid contamination. GC grade reagents were used throughout the study. For each batch of analysis, method blanks and spiked samples at fortification level of 0.05 mg /kg were used for quality control checks.

All reagents used during the analysis were exposed to the same extraction procedures. Solvents used were run to verify for any interfering substances within the runtime. In all batches of pesticide residues analysis, reagent blanks and samples to be analyzed were fortified with mixed OPPs, OCPs and synthetic pyrethroid standards for quality control checks. All the samples were analyzed in triplicates. Matrix match Calibration curves were run with each batch of samples to check that the correlation coefficient was kept above r^2=0.99.
Confirmation was made by GC-MS in the selected ion monitoring mode (SIM) using one target ion and the qualifying ions (Table 3.1).

The glassware was soaked in soapy water for overnight after that they were thoroughly washed with detergent. After washing, the glass wares were rinsed several times with tap water followed by deionized water. Finally, rinsed with acetone and then dried in an oven at 300 $^{\circ}$ C for overnight. The glass wares were then removed from the oven and allowed to cool down and rinsed with the same solvent to be used in the analysis.

Table 3. 1 The retention time, qualifier ions and selected quantification ions obtained for the studied pesticides.

S/N	pesticides	Quantification ions(m/z)	Qualifier ions (m/z)	Molecularmass
1.	α- BHC	181	219,183	288
2.	Diazinon	179	173,152,199	304
3.	δ-BHC	181	219,183	288
4.	Heptachlor	272	237,100	370
5.	Malathion	127	173,125	330
6.	Aldrine	263	293,66	365
7.	Chlorpyrifos	197	314,97,199	349
8.	γ-chlordane	373	375,377	406
9.	α-Endosulphan	195	241,170	404
10.	p,p DDE	246	318,176	316
11.	Dieldrin	79	263,277	378
12.	Endrin	263	245,279	378
13.	p,p DDD	235	165,237	318
14.	Endosulphan sulphate	272	229,237	420
15.	p,p DDT	235	165,237	352
16.	Metoxychlor	227	228,213	344
17.	λ- cyhalothrin	181	197,208	449
18.	Cyfluthrin	206	163,199,281	434
19.	Cypermethrin	163	181,165	415
20.	Fenvalerate	125	207,167,281	419
21.	Deltamethrin	253	207,281,73,	503

3.4.5 Verification of analytical procedure

The extraction method used, QuEChERS, had previously been validated by Lehotay et al. (2007) and verification was performed in terms of precision, recovery , limit of detection (LOD), limit of quantification (LOQ) and linearity. Blank apple, strawberry, lettuce and tomato samples previously check for the presence of pesticides from collected samples were used for the method optimization and verification.

Recovery and precision were determined by spiking pesticide standards in comminuted blank tomato, apples, strawberry and lettuce samples five times at three concentration levels 0.05, 0.1 and 0.2 mg/ kg (in the case of OPPs and pyrethroids the levels were 0.25 ,0.5 and 1 mg/kg). The recovery of each compounds were estimated by comparing the peak area of an extracted spiked blank matrix to those counterparts from the matrix matched calibration curves. The precision of the method was judged in terms of repeatability. Repeatability was calculated as the percentage relative standard deviation (% RSD) of results obtained for each analyte after the replicate (n = 5) analyses under the same conditions and on the same day (Park et al., 2016).

The recovery was determined using the formula:

$$\% \text{ Recovery} = \frac{\text{Pesticide recovered from fortified sample}}{\text{Amount of pesticide added}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

The LOD and LOQ were calculated using a signal to noise (S/N) ratio of 3 and 10, respectively. Linearity was evaluated using matrix-matched calibration, spiking apple, tomato, strawberry and lettuce blank samples at seven concentration levels (0.001, 0.01, 0.02, 0.04, 0.07, 0.1 and 0.2 for OCPs) mg/L, for OPPs and pyrethroids, the same number of levels but with five times higher at each level. The same extraction procedure as sample was applied for matrix match calibration.

3.4.6 Extraction and clean up

Pesticides were extracted from apple, tomato, strawberry, and lettuce using the **QuEChERS** (**Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged and **S**afe) methodology. The procedures were based on Association of Official Analytical Chemists method 2007.01 (Lehotay et al., 2007). The procedure is described briefly as shown in Figure 3.5. The frozen samples thawed until it reached room temperature. 10.0 ± 0.1 g of representative sample was weighed in a 50 ml

polypropylene centrifuge tubes. A reagent blank sample, which was the negative control, was prepared by pipetting 10 ml of deionized water into a 50 centrifuge tube. Spiking was done for quality control samples at 0.05 mg/kg. Next, 10 ml acetonitrile was added with 4 g anhydrous magnesium sulfate ($MgSO_4$) for water removal, and 1 g anhydrous sodium acetate for phase separation. The tube was immediately vortexed for 1 min to prevent the formation of crystalline agglomerates during $MgSO_4$ hydration. Afterward, the tubes were closed and centrifuged at 5000 rpm for 5 minutes. A cleanup procedure is usually carried out to remove co-extracted compounds that may cause interference in the chromatographic determination or be detrimental to the analytical instrumentation. Dispersive-solid-phase extraction (dispersive-SPE) clean-up was done to remove organic acids, excess water, pigment and other components with a combination of primary secondary amine (PSA) sorbent, graphitized carbon black (GCB) and anhydrous magnesium sulfate ($MgSO_4$). Following extraction as shown in figure 3.4, 6 ml aliquot of the supernatant was transferred into 15 ml polypropylene centrifuge tubes containing 300 mg PSA, 900 mg $MgSO_4$ and 100, 15, 60, and 60 mg GCB was added to lettuce, apple, strawberry and tomato matrices based on the amount of pigment, respectively. The tubes were closed and vortexed for 1 min and centrifuged again for 5 min at 5000 rpm. An aliquot of 4 ml upper acetonitrile layer was transferred into a 10ml glass tube. Then, the extract was evaporated to dryness under slow stream nitrogen at 35 ^{o} C. Finally, the residue was re-dissolved in 1 mL n-hexane, sonicated for 1 minute, filtered through 0.22 µm nylon filter membrane, and two µL of the extract was injected into the gas chromatography system.

Before clean up after clean up

Figure 3. 4 Clean up procedure

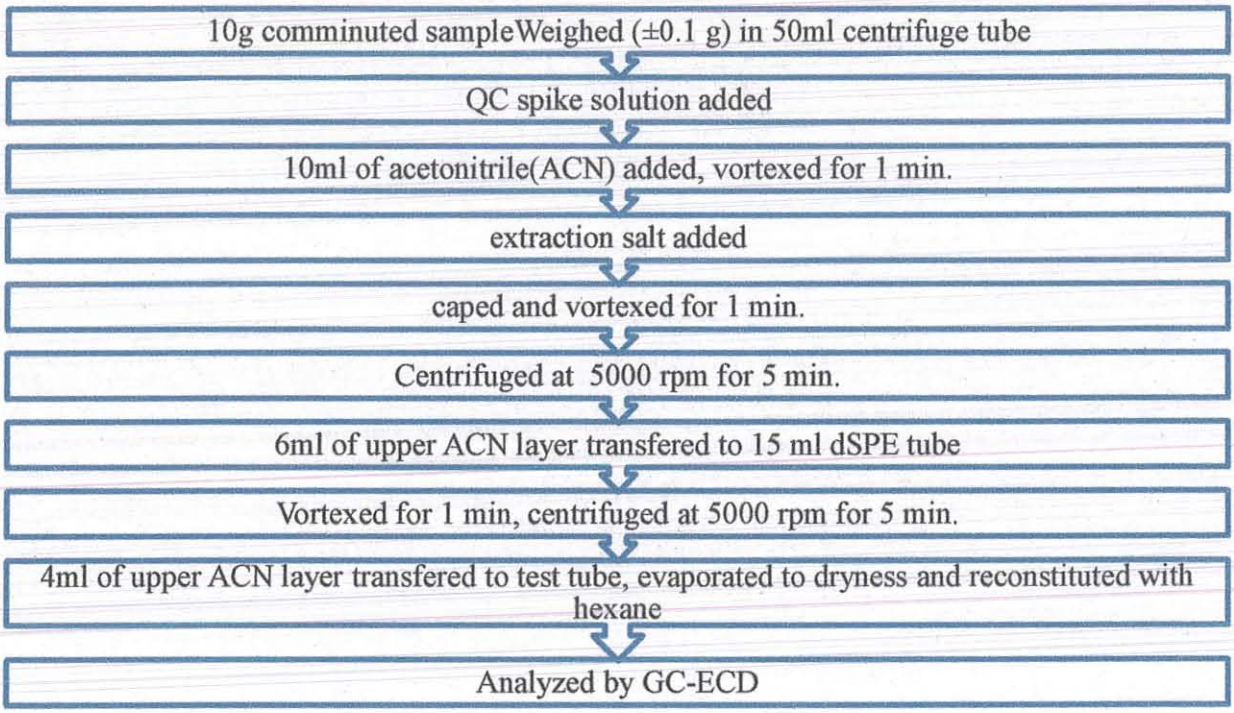


Figure 3. 5 Flow chart of extraction procedure

3.4.7 Quantification and identification of pesticides

An external standard method was used for determining the quantities of residues in the sample extracts. A standard mixture containing known amounts of pesticides was run and the response of the detector for each compound was determined under the same condition. The area of the corresponding peak in the sample was compared with that of the known standard.

Pesticide residues in the extracts were identified when the retention times (within 0.5%) matched those of the reference standards.

For pyrethroids, the quantification of these compounds was performed by summing the peak areas of their isomers. They contain two or three chiral centers, making them a family of pesticides with stereoisomers. Therefore, multiple peaks were observed for several of the pyrethroids, corresponding to the separation of their diastereoisomers. Deltamethrin, fenvalerate, lambda-cyhalothrin and cypermethrin were resolved using two peaks, while four peaks were observed for cyfluthrin (Li et al., 2012).

For organochlorine pesticide, the sum of alpha- benzene hexachloride (α-BHC) and delta - benzene hexachloride (δ-BHC) expressed as lindane. The sum of alpha-endosulfan and endosulfan - sulfate expressed as endosulfan. DDT (sum of p,p′-DDT, p-p′-DDE and p,p′-DDD) expressed as ∑ DDT.

Calculation of final result was performed using the following equations.

Y = (a*x) / (b*z).....2

Where,

Y is grams of sample equivalent per milliliter of extract, **a** is the amount of sample analyzed (g), **b** is the volume of solvent added to extract the sample (mL), **x** is the amount of the cleaned extract taken after evaporation until dryness (mL), and **z** is the amount of hexane added for solvent exchange (mL).

3.5 Washing experiment

Fruits and vegetable samples for which pesticide residues were detected (lindane (α-BHC and δ-BHC), heptachlor, γ-chlordane, ∑DDT, λ-cyhalotrhin, cyfluthrin, cypermethrin and fenvalerate), were subjected to washing experiments. Samples taken for wash treatment were washed with running tap water for two minutes by gentle rubbing with hands to simulate the normal cleaning

procedure held at house hold level with majority our society, as described by Wanwimolruk et al. (2015). Figure 3.6 shows washing of lettuce sample in the Ethiopian conformity assessment enterprise (ECAE) laboratory. The washed samples were put on tissue paper just to remove the excess of the water. Subsequently, the fruits and vegetable samples were extracted, cleaned up in a similar manner as the fresh fruits and vegetables, were processed and analyzed.

Figure 3. 6 Washing of lettuce samples

3.6 Knowledge, attitude and practice (KAP) to wards pesticide residue

The sampling procedure for KAP used was probability sampling specifically simple random sampling so as to minimize bias in order to obtain representable data. The data were collected through interview. A total of fifty five consumers' from different sub city of Addis Ababa were randomly selected and interviewed by using structured questionnaire. The questionnaire contained four sections. The first section consisted of general information like age, sex, educational status etc. The second section designed to assess knowledge of respondents about pesticides. The third section had questions related to consumers' beliefs about the health effects of pesticides. The fourth section consisted of questions related to practice of consumers (decision, action) that demonstrated knowledge and attitudes towards pesticides (Appendix D).

3.7 Statistical Analysis

All analysis was conducted in triplicates and the data was averaged and expressed in the form of mean ± standard deviation. Two statistical analysis tests were used pair-difference t-test and analysis of variance (ANOVA). The pair-difference t-test is simply used to test two independent populations have different mean values on some measure. Paired t-test analyses were performed to compare between residuals in washed and unwashed fruits and vegetables. The analysis of variance (ANOVA) was used to compare single factors. Descriptive statistics and Microsoft excel were also used to summarize the data. The data was reported as mean ± SD and the levels were considered significantly different at $P < 0.05$.

Chapter four

4 Results and discussion

4.1 Verification result of the analytical method

The results corresponding to the mean recovery and precision in terms of relative standard deviation (RSD) at both fortification levels are summarized in Table 4.1.

Table 4. 1 Method verification parameters of OC, OP and pyrthriod pesticides in strawberry, tomato, apple and lettuce samples.

Pesticides	Spiking level (mg/kg)	Apple		strawberry		Lettuce		Tomato	
		R%±SD	RSD	R%± SD	RSD	R%±SD	RSD	R% ±SD	RSD
α-BHC	0.05	95.7 ±1.9	1.7	85.4±5.4	6.3	97.6± 4.9	5	103.5±4	3.9
	0.1	107.4±3.9	3.9	90.0±7.9	8.8	96.1 ±2.4	2.5	92.0 ±1.9	2.1
	0.2	98.4±0.9	0.9	103.1±1	1.3	101.1± 0.7	0.6 0.7	103.7±2.5 0.6	2.5
Diazinon	0.25	99 ± 1.2	1.2	103.3± 0.9	0.8	100.3 ±0.6	0.6	102.9 ± 5.6	5.5
	0.5	109.4±2.6	2.5	88.9±4.2	4.7	106.4±3.6	3.4	116.4 ±6.6	5.6
	1	101.2±5.1	5.2	91.1±6.9	7.6	96.7±3.1	3.2	103.6±5.5	5.3
δ-BHC	0.05	105.3±3.9	4.1	83.3±4.2	5	96.53.4	3.5	103.9±4.1	4.0
	0.1	97.3±7.5	7.2	92.0±6.9	7.5	93.4±15	1.5	98.3 ±5.2	5.3
	0.2	100.3±1.8	1.8	103.7±05	0.4	101.8 ±0.4	0.4	96.6±4.1	4.3
Heptachlor	0.05	94.3±1	0.9	84.1±4.9	5.8	95±3.9	4.1	99.4±7.7	7.8
	0.1	104±3.8	3.8	91.1±7.2	7.9	91.9±3.6	3.9	97.5±4.3	4.4
	0.2	99.3±1	1	103±1.2	1.2	102.3±0.9	0.9	104.9±48	4.6
Aldrin	0.05	100.6±2.5	2.5	87.4±1.3	1.5	95.9±4.0	4.2	103.7±81	7.8
	0.1	106.1±3.1	3.2	88.4±1.3	1.3	93.9±2.4	2.6	103.3±53	5.1
	0.2	98.5±0.9	0.9	103.5±04	0.4	101.8 ±0.5	0.5	103.8±56	5.3
Chlorpyrifos	0.25	100.7±0.9	0.9	103.3±14	1.4	101.3±1.4	1.3	103.5±50	4.8
	0.5	99±1.3	1.3	84.1±4.6	5.5	100.6± 6.6	6.6	102.2±80	7.8
	1	97.5±3.3	3.4	90.0±8.2	9.1	94.4±4.6	4.4	103.5±49	4.7
Malathion	0.25	99.4±0.7	0.7	102.5±15	1.5	100.3±1.1	1.1	99.9±1.6	1.6
	0.5	108±1.3	1.3	71.2±6.9	9.7	103.9±6.8	6.6	109.6±62	5.7
	1	100.3±2.7	2.5	79.2±3.5	4.4	97.1±4.5	4.6	104.220	1.9
γ-Chlordane	0.05	98.3±2.2	2.2	85.2±5.2	6.1	93.4±3.6	3.9	100.8±75	7.5
	0.1	97.8±3.6	3.7	90.2±7.9	8.8	91.6±2.8	3.1	104.9±58	5.5
	0.2	100.6±1	1	103.3±14	1.3	102.5± 0.7	0.7	103.9±58	5.6
α-endosulphan	0.05	98.3 ± 2.2	2.2	80.8±5.9	7.3	94±3.7	3.9	102.4±66	6.5
	0.1	97.8 ± 3.6	3.7	89.7±8.6	9.6	91.5±3	3.3	104.8±42	4.0
	0.2	100.6 ± 1	1	103.7±15	1.4	102.4±0.7	0.7	103.8±50	4.3
p,p DDE	0.05	98.7±2.5	2.5	83.4±6.3	7.5	99.4± 4.1	4.2	98.7±2.5	2.5
	0.1	97.7 ± 3.8	3.8	90.1± 7.8	8.7	92.7 ± 5.5	5.9	97.7±3.8	3.8
	0.2	100.6 ± 1	1	103.4±14	1.4	101.9± 1.4	1.4	100.6±1	1
Dieldrin	0.05	97.1±1.7	1.7	80.2±6.4	7.9	91.5± 3.9	4.3	100.7±72	7.1
	0.1	97.9±3.1	3.2	89.2±9.0	10.0	89.8±2.8	3.1	104.3±52	5.0
	0.2	100.7±0.8	0.8	103.1±18	1.7	103 ± 0.7	0.7	103.0±46	4.5
Endrin	0.05	98.6 ± 1.6	1.6	81.3±4	4.9	93.1± 3.4	3.6	99.4±7.1	7.1
	0.1	97.4±3.4	3.6	90.1±8.6	9.6	89.5 ± 3.3	3.7	104.3±52	5.0
	0.2	100.7±0.9	0.9	118.5±15.9	13.4	103 ± 0.9	0.8	103.8±55	5.3
p,p DDD	0.05	98.7±1.3	1.2	83.4± 6.3	7.5	94.4 ± 3.9	4.2	101.3±81	8.0
	0.1	94.5±3.1	3.2	90.1±7.8	7.8	90.9 ± 2.2	2.5	105.8±52	4.9
	0.2	101.4±0.8	0.8	103.4±14	1.4	102.6±0.6	0.6	103.3±55	5.3
p,p DDT	0.05	87.2 ± 2.5	2.8	75.3 ±8.2	10.9	90.6±3.0	3.3	92.4 ±5.9	6.4
	0.1	93.1±2.4	2.4	88.0±101	11.5	90.8±5.3	5.8	99.8 ±3.3	3.4
	0.2	102.5± 0.7	0.7	102.7±17	1.2	102.8 ±1.3	1.3	102.6±19	1.9

Pesticides	Spiking level (mg/kg)	Apple		strawberry		Lettuce		Tomato	
		R%±SD	RSD	R%± SD	RSD	R%±SD	RSD	R% ±SD	RSD
Endosulphan sulphate	0.05	104.6±3.5	4	81.3± 6.5	8	91.8 ± 4.4	4.8	101.6±2.3	2.3
	0.1	96.5±3.4	3.6	99.7±7.8	7.9	93.6 ± 5.6	6	99.7±5.6	5.6
	0.2	100.5±0.9	0.9	116.6±16.6	14.2	102±1.7	1.7	96.0±4.9	5.1
Metoxychlor	0.05	89.5±4.9	5.2	78.1±6.8	8.7	92±4.0	4.5	93.0±8.4	9.0
	0.1	97.8±2.4	2.4	94.6±10.8	11.4	92.8±49	5.3	99.8± 3.7	3.7
	0.2	101.2±0.8	0.8	109.9±0.95	13	102.2±1.3	1.3	102.5±2.6	2.5
λ-cyhalotrhin	0.25	101.35±0.7	0.7	103.55±7.05	0.95	87.95±8.9	10.7	102.3± 3.2	3.1
	0.5	96.8±4.55	4.6	80.65±7.05	8.9	86.65±3.5	4.2	99.8± 8.2	6.1
	1	95.1±2.4	2.55	94.2±7.8	8.3	101.95±1.05	1.05	104.8±4.45	4.3
Cyfluthrin	0.25	101.6±0.625	0.625	111.5±10.9	9.775	102.1±1.15	1.075	99.8±1.0	1.0
	0.5	98.6±2.15	2.2	83.3±4.4	5.275	92.175±4.0	4.35	99.4±5.6	6.0
	1	93.7±2.35	2.475	94.3±8.7	9.2	92.075±4.5	4.925	102.9±1.9	1.9
Cypermethrin	0.25	100.95±0.8	0.8	102.95±1.4	1.4	99.05±3.3	3.35	100.3±2.5	2.5
	0.5	98.35±2.85	2.95	84.45±5.2	6.1	104.55±8.1	7.85	101.4±6.0	5.9
	1	96.7±3	3.2	94.2±7.6	8.1	90.25±6.05	7.25	103.95±3.55	3.4
Fenvalerate	0.25	101.35±0.8	0.8	103.7±0.75	0.75	101.7±1.05	1.1	99.4±1.2	1.2
	0.5	98.6±2.95	3	82.02±5.75	7	91.35±4.5	4.95	97.3±5.0	5.1
	1	94.9±3.25	3.4	91.5±8.0	8.8	90.2±7.55	8.5	103.3±2.6	2.55
Deltamethrin	0.25	101.4±0.75	0.75	102.4±1.2	1.15	100.65±1.6	1.55	98.5±2.6	2.7
	0.5	99.85±3.55	3.6	88.5±4.8	5.45	94.55±6.1	6.45	96.5 ±6.9	7.3
	1	94.5±3	3.15	94.8±3.6	2.7	92.3±9.15	10.4	100.7±3.3	3.4
R recovery; RSD relative standard deviation; SD standard deviation									

According to European Commission regulation, the recovery rate (%) between 70% - 120% and RSD<20% can be considered good recoveries and RSD (Alimentarius, 2017; SANTE, 2015). All of the verification results fulfilled the criteria for the acceptance mentioned above.

Identification of pesticides were done according to their retention time, which was obtained after running individual pesticides standard at 100 µg/L and mixed standard at 50 mg/L concentration level. Figure 4.1 - 4.5 shows chromatogram of solvent, method blank, apple sample free of pesticide, apple sample contaminated with pesticides and apple sample spiked at concentration level of 0.05 µg/kg.

Ion chromatogram obtained after injecting 2 µL of extracted sample confirmed using two target ions (Appendix-B).As an example, figure 4.6 shows the ion chromatogram of the GC-MS analysis of strawberry sample contaminated with γ-chlordane pesticide.

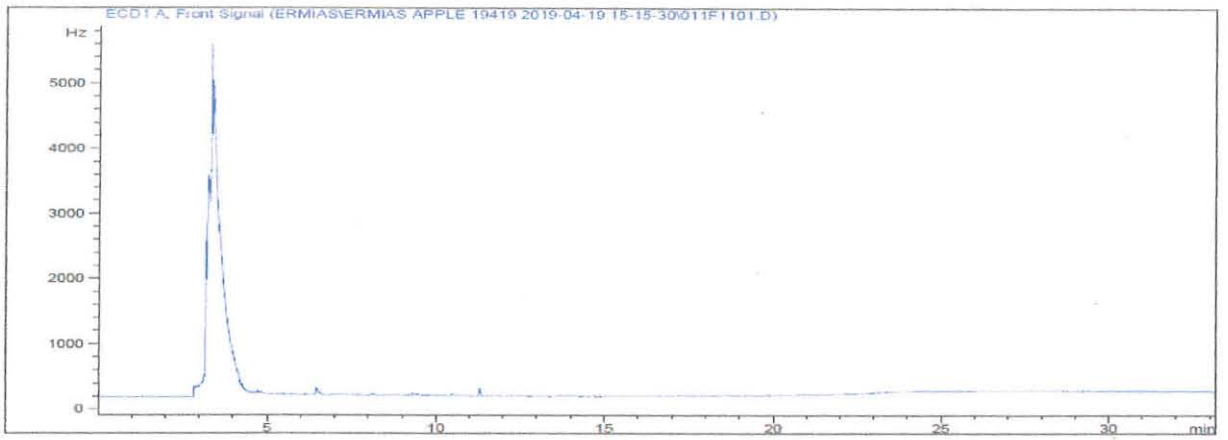


Figure 4. 1 GC/ECD chromatogram of solvent

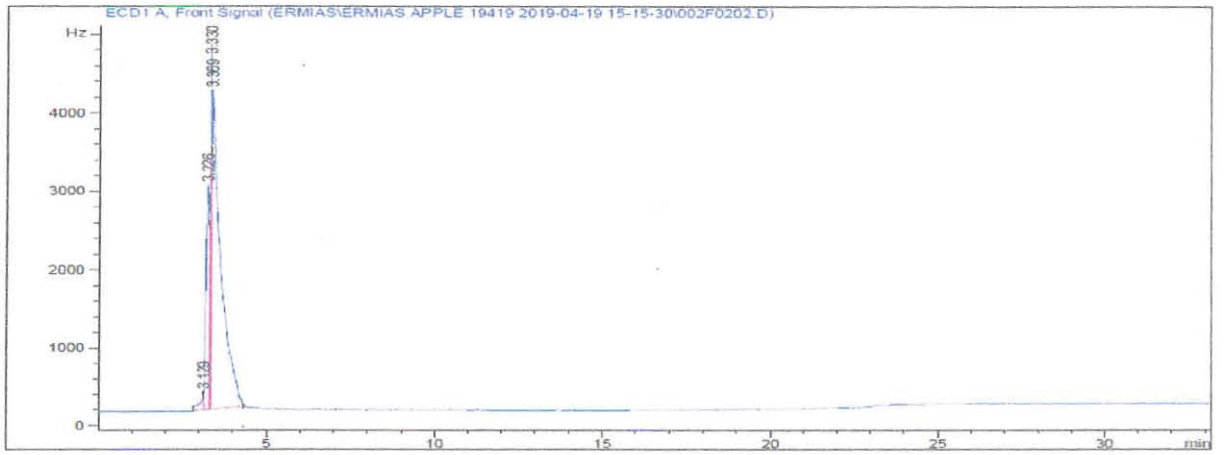


Figure 4. 2 GC/ECD chromatogram of method blank

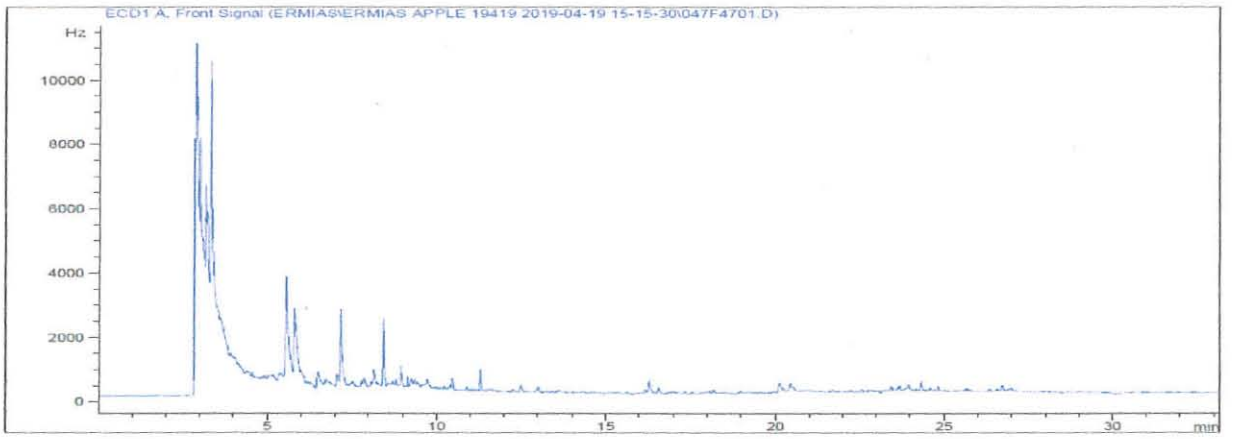


Figure 4. 3 GC/ECD chromatogram of apple extract blank

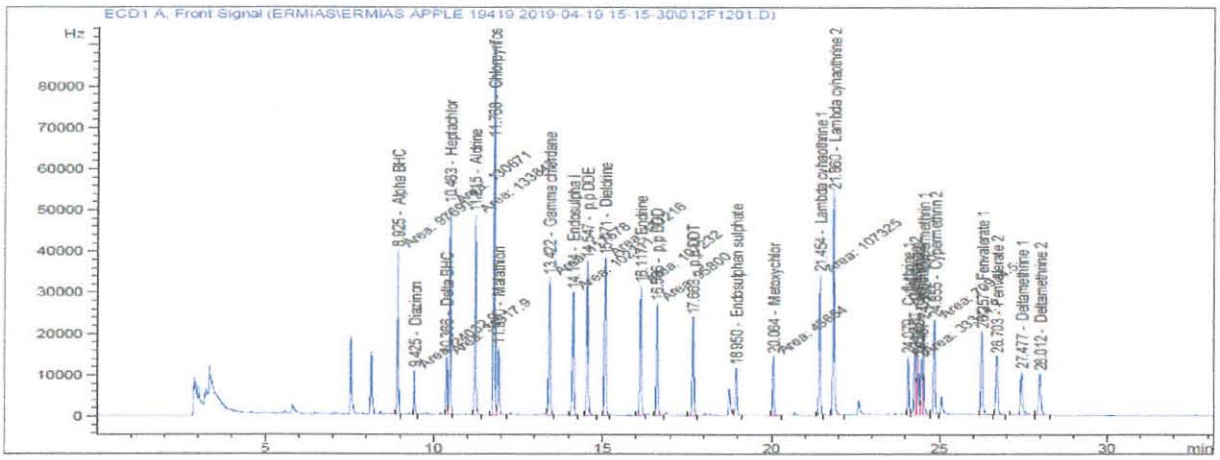


Figure 4. 4 GC/ECD chromatogram of apple sample spiked at 0.05 mg/kg

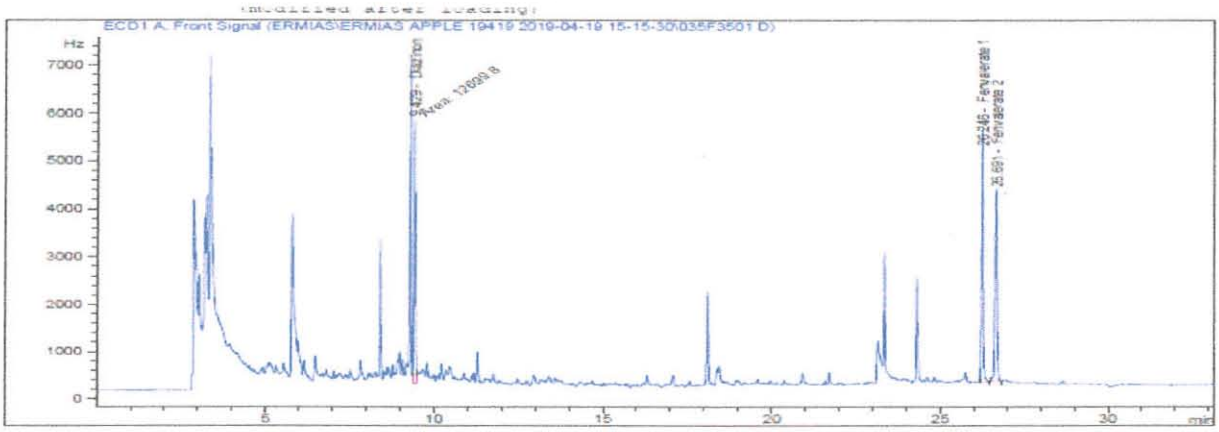
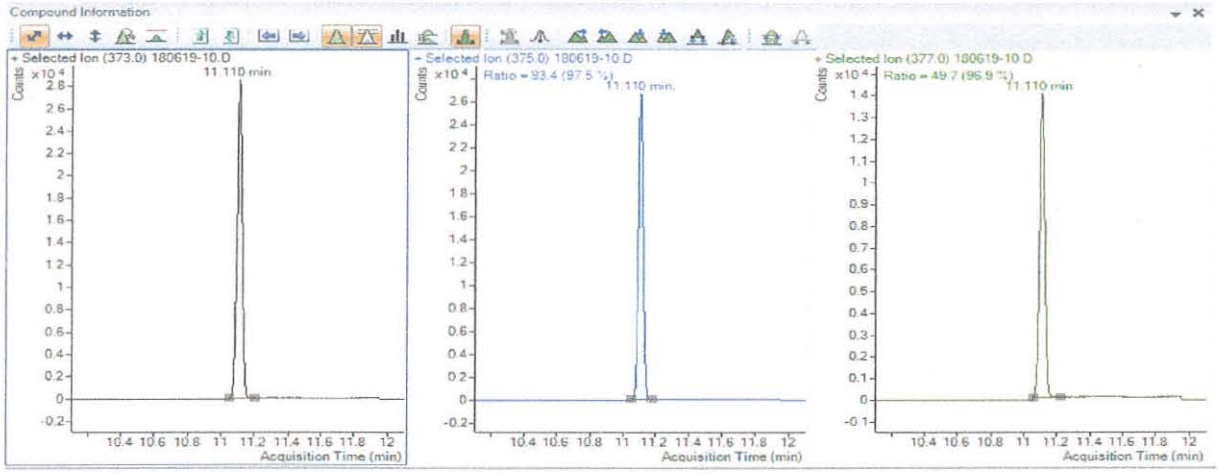


Figure 4. 5 GC/ECD chromatogram of apple sample

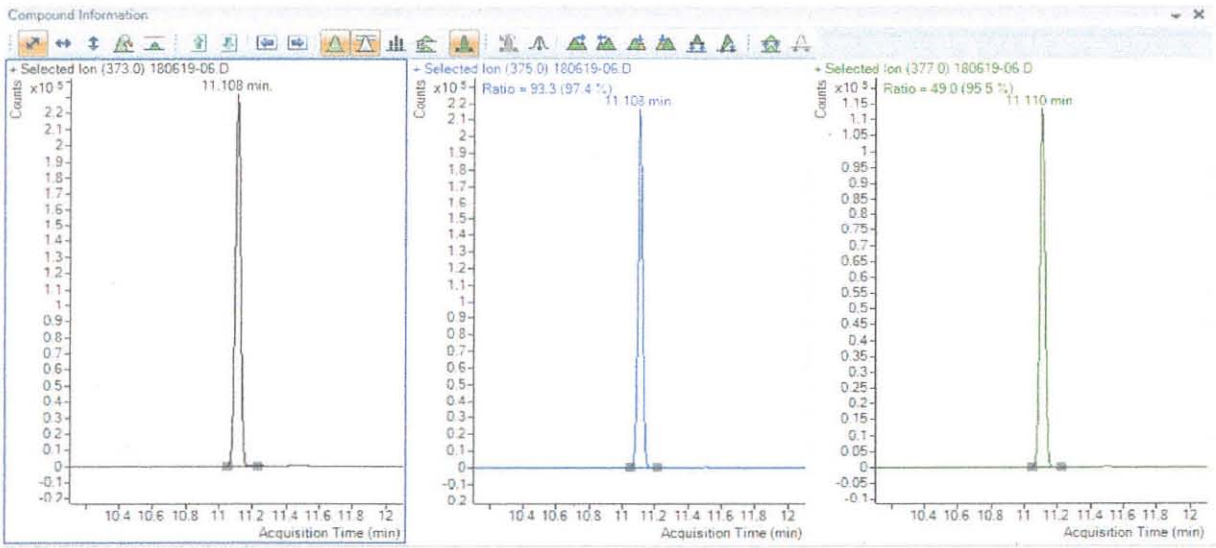
As observed from the figure the chromatogram of hexane and method blank were free from interfering compounds.

A matrix-matched calibration curve was used for in each sample to prevent matrix effect. A matrix-matched calibration curve was constructed for each pesticide at seven calibration levels having concentration range of 0.001 to 0.2 mg/kg (in the case of OPPs and pyrethroids the range were 0.005 to 1 mg/kg). Good linear relationships were achieved with linear regression coefficients (r^2) of 0.99 or higher over the examined concentration range, as shown in Appendices A. Figures 4.7 show some examples of calibration curves in lettuce sample. Exact matching of the matrix for a sample type is one of the approaches that can be used to resolve

matrix effects and obtain accurate results (Lehotay et al., 2005) and a new alternative to matrix-matching could be the use of analyte protectants.

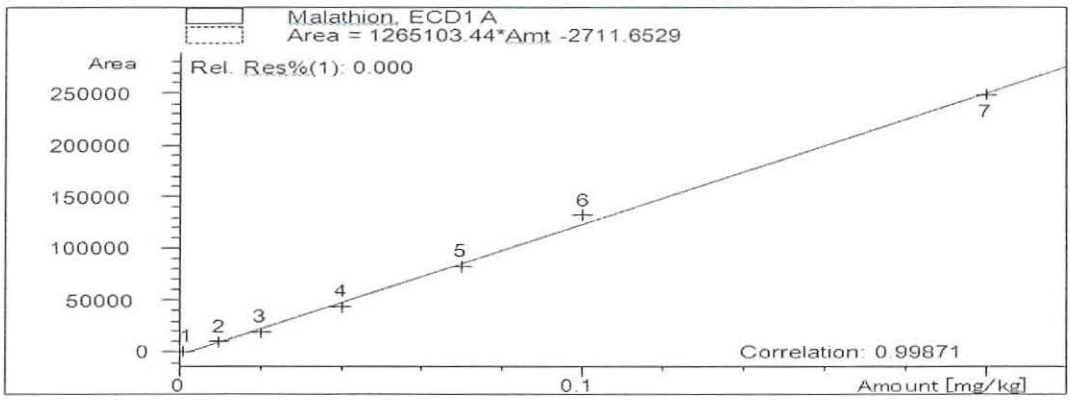


a

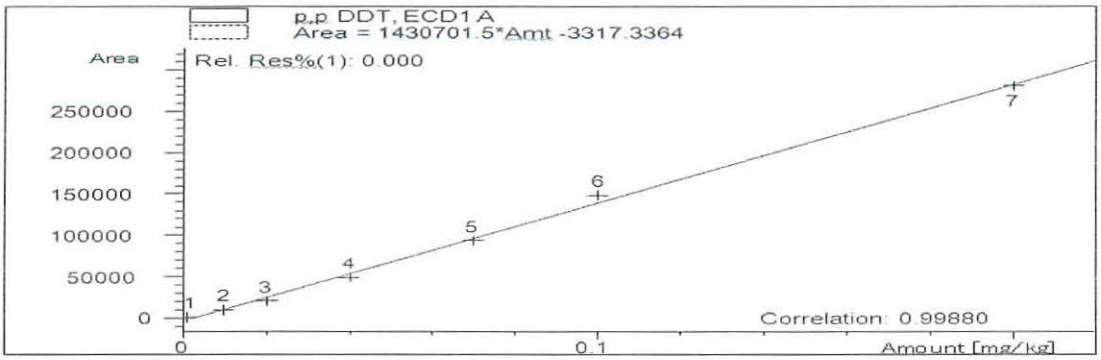


b

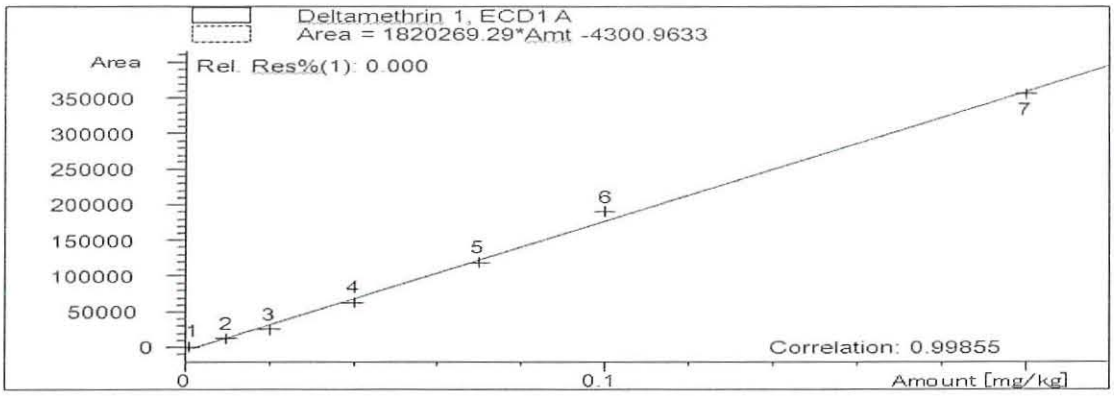
Figure 4. 6 GC/MS chromatogram: (a) Ion chromatogram of strawberry sample contaminated with γ-chlordane pesticide residue; (b) Ion chromatogram of γ-chlordane pesticide Standard.



a



b



c

Figure 4. 7 Calibration curve of pesticides in lettuce: (a) malathion; (b) p,p DDT ; (c) deltamethrin

Determination of LOD and LOQ can be done by Signal- to-noise, blank determination and linear regression. However in this research signal -to- noise determination were used to determine LOD and LOQ (Shrivastava & Gupta, 2011).The LOD and LOQ for 21 pesticides are provided in Table 4.2. The LOD and LOQ ranged from 0.08 to 3.50 µg/kg and 0.41 to 17.52 µg/kg, respectively. The results showed that the applied method was reliable for the analysis of the pesticide residues in this study.

Table 4. 2 Verification parameters obtained for the optimized method: limit of detection (LOD), limit of quantification (LOQ) and retention time (RT)

S/N	PESTICIDES	LOD (µg/kg)	LOQ (µg/kg)	RT (min)	
				GC-ECD	GC-MS
1	α- BHC	0.18	0.90	8.909	7.488
2	Diazinon	2.19	10.95	9.409	8.157
3	δ-BHC	0.19	0.95	10.345	8.321
4	Heptachlor	0.15	0.77	10.440	9.117
5	Malathion	1.84	9.20	11.188	9.405
6	Aldrine	0.16	0.79	11.740	9.749
7	Chlorpyrifos	0.63	3.13	11.882	9.756
8	γ-chlordane	0.20	0.98	13.384	11.109
9	α-Endosulphan	0.21	1.05	14.074	11.449
10	p,p DDE	0.17	0.87	14.508	12.044
11	Dieldrin	0.08	0.41	15.030	12.188
12	Endrin	0.20	1.00	16.076	12.899
13	p,p DDD	0.13	0.67	16.558	13.460
14	Endosulphan sulphate	0.43	2.15	17.624	14.769
15	p,p DDT	0.54	2.69	18.908	14.898
16	Metoxychlor	1.33	6.64	20.028	17.036
17	λ- cyhalothrin	1.93	9.66	21.828, 21.4218*	18.930
18	Cyfluthrin	1.70	8.52	24.344, 24.241, 24.051, 24.437*	21.371
19	Cypermethrin	0.94	4.68	24.823,24.499*	21.925
20	Fenvalerate	1.55	7.77	26.661,26.219*	23.394
21	Deltamethrin	3.50	17.52	27.965,27.433*	24.514

*Two or more retention time observed for synthetics pyrethriod

4.2 Samples contaminated with pesticides residues

The percentage of samples of fruits and vegetables with pesticide residues are presented in the Table 4.3. The percentage of samples of fruits and vegetables with pesticide residues in Lettuce, strawberry, tomato and apple were 100%, 90%, 90%, 70% respectively and details are discussed below.

Table 4. 3 Percentage of samples with one or more pesticide residues

Vegetable/fruit	Scientific name	Number of samples	% with one or more residues
Lettuce	*Lactua sativa*	10	100
Apple	*Malus domestica*	10	70
Strawberry	*Fragariaxananassa*	10	90
Tomato	*Lycopersican esculentum*	10	90
Total		40	

4.2.1 Occurrence of organochlorine pesticides

Table 4.4 show the various mean concentration of organochlorine pesticide residues detected in samples. In all, a total of six organochlorine pesticides were detected and these include lindane (α-BHC and δ-BHC), heptachlor, endosulfan (alpha-endosulfan and endosulfan-sulfate), DDT (p,p-DDT, p-p-DDE and p,p-DDD), methoxychlor and γ-chlordane. Aldrin, dieldrin and endrin were not detected in any of the samples. The sample which recorded a greater number of organochlorine pesticides residues was lettuce followed by strawberry with apple and tomato recording fewer number of organochlorine pesticides residues. The concentration of the organochlorine in the fruits and vegetables was not varied significantly ($p > 0.05$ or $p = 0.17$, Appendix-D) suggesting a common route of contamination.

The highest concentration of organochlorines in lettuce was recorded for lindane (α-BHC and δ-BHC) with mean concentration of 10.83 µg/kg while DDT and γ-chlordane recorded lowest mean concentration of 3.38 µg/kg and 3.86 µg/kg respectively. Amoah et al. (2006) reported that from 180 fresh vegetables including lettuce from 9 major markets of Ghana were having a positive result on lindane (300 µg/kg).Most of the residues recorded exceeded the MRLs for consumption. This value was high as compared to our results. This is due to that restricted use of lindane in Ghana for the control of capsids on cocoa, stem borers in maize, and pests on coffee.

The residue level from Addis Ababa markets are in agreement with the findings of Kolani et al. (2016), from 150 lettuce samples collected and analyzed linden were found in all samples.

The high levels of organochlorine pesticide residue in lettuce samples from Addis Ababa suggest that farmers who supply fruits and vegetables to these markets either they do use organochlorine pesticide in significant quantities or due to their persistence nature in the environment.

In fact that, the use of most organochlorine pesticides has been banned or restricted in Ethiopia under the Stockholm convention due to high levels of persistence in the environment and toxicity to non-target organisms (Yohannes et al., 2014). The illegal use may be one reason and the other reason is due to their long-term stability in environment. Pesticide residues remained in aquatic resources, plants, and weeds are transferred to the food chain (Carvalho, 2006). Similar problem also existed in other countries that banned pesticides were detected in vegetables (Fang et al., 2015; Swarnam & Velmurugan, 2013).The presences of OCPs residues may be related to their previous use not to new inputs after they were banned.

In Ethiopia, practically, DDT was used to control the mosquito in public health programs (van den Berg, 2009) from where it could enter the agricultural soils and water systems and possibly find its way into crops.

The highest concentration of 10.68 µg/kg was obtained for chloridane (γ-chloridane), followed by a concentration of 7.3 µg/kg for lindane (α-BHC and δ-BHC) respectively in strawberry sample. As many organochlorine pesticides like chlordane and lindane have been banned in many countries throughout the world, but they have remained in the environment where they continue to be incorporated into plant biomass.

In general lowest concentration of OCP residues was recorded for the detected pesticide in apple and tomato samples. However, the monitoring of pesticides residues in food is very important to protect the public health.

Table 4. 4 Concentrations (μg/kg) of organochlorine pesticide residue

Pesticides	∑Lindane	Heptachlor	γ-Chlordane	∑Endosulphan	∑DDT	Metoxychlor
Lettuce	10.83 ± 3.94^{a}	6.82 ± 3.03^{a}	3.86 ± 0.21^{b}	5.81 ± 0.77^{a}	3.38 ± 0.28^{b}	6.44 ± 0.73^{a}
Apple	3.91 ± 1.5^{b}	3.96 ± 0.01^{c}	2.19 ± 0.01^{b}	$<LOD^{c}$	2.40 ± 0.1^{a}	$<LOD^{b}$
Strawberry	7.33 ± 0.35^{b}	$<LOD^{d}$	10.58 ± 0.26^{a}	3.26 ± 0.07^{b}	1.58 ± 0.03^{a}	$<LOD^{b}$
Tomato	3.99 ± 0.18^{b}	3.57 ± 0.41^{b}	$<LOD^{b}$	3.56 ± 0.08^{b}	2.98 ± 0.02^{a}	$<LOD^{b}$

Values are mean± SD, different letters in the same column represent statistically significant difference ($P<0.05$), < LOD less than limit of detection

4.2.2 Occurrence of synthetic pyrethroid pesticides

The results obtained from the analysis of samples are shown in table 4.5. Total samples analyzed show the presence of four synthetic pyrethroids. The four synthetic pyrethroids pesticides detected were λ-cyhalotrhin, cyfluthrin, cypermethrin and fenvalerate. The most predominant synthetic pyrethroids pesticide in lettuce, strawberry, tomato and apple was fenvalerate with mean concentrations of 21.1 μg/kg 26.17 μg/kg, 64.9μg/kg and 169.9μg/kg respectively (table 4.5).

The highest concentration of fenvalerate pesticide residue was 169μg/kg found in imported apple sample. The reason for this may be different agricultural practices adopted by farmers and also accessibility of the pesticides. For instance higher quantities of pesticides may be applied in areas of massive pest attack and the frequency of pesticide application may be influenced by climatic conditions such as temperature and rainfall. Deltamethrin was not detected in any of the analyzed samples.

Fenvalerate was detected with a mean concentration of 21.1μg/kg for lettuce sample. This value is far lower than those reported by Amoah et al. (2006), carried out a study on pesticide residues levels in a total of 180 vegetable samples (lettuce, cabbage and spring onion) which were randomly collected from Ghanaian cities, who obtained 10-1400 μg/kg in lettuce. From the

results, it can be deduced that synthetic pyrethroids levels detected in lettuce, strawberry and tomato were below the maximum residue limits (MRLs) set forth by the FAO/WHO Codex Alimentarius Commission and EU commission.

The presence of synthetic pyrethriods in tomatoes and apples are an indication of the use of legally permitted pesticides by farmers.

According to Pakvilai et al. (2012), from the report on the level of synthetic pyrethroids pesticide residue in fruit and vegetable from a different city of Thailand high incidence of the positive sample observed. The result shows the highest mean value of cypermethrin in fruits at 854µg/kg, while deltamethrin shows the highest mean value in vegetables at 874µg/kg.As compared to the above report the occurrence of SPPs in apple, strawberry, tomato and lettuce from Addis Ababa markets is at the lower level in both the number of positive samples and maximum concentration value, i.e. 188.99µg/kg in this study.

Table 4. 5 Concentrations (µg/kg) of synthetic pyrethroids pesticide residue

Pesticides	λ-cyhalotrhin	Cyfluthrin	Cypermethrin	Fenvalerate	Deltamethrin
Lettuce	< LOD[b]	< LOD[b]	< LOD[b]	21.10 ±1.6[b]	< LOD
Apple	< LOD[b]	22.26±0.9[a]	27.65±0.8[b]	169.59±2.3[a]	< LOD
Strawberry	< LOD[b]	< LOD[b]	11.67±1.19[b]	26.17±0.96[b]	< LOD
Tomato	55.54±0.47[a]	< LOD[b]	188.99±13.5[a]	64.9±17.2[b]	< LOD

Values are mean± SD, different letters in the same column represent statistically significant difference (P<0.05), < LOD less than limit of detection

4.2.3 Occurrence of organophosphate pesticides

Only diazinon was detected in apple sample with a mean concentration of 51.67µg/kg (table 4.6). Chlorpyrifos and malathion were not detected in any analyzed sample. The absence of organophospahtes in tomato, lettuce and strawberry suggests either proper use of these pesticides or farmers do not use these pesticides in fruit and vegetable production.

Similar result was reported by Jallow et al. (2017), on monitoring program conducted between September 2015 to August 2016 on 150 fruits and vegetable samples obtained from Kuwait

markets it was reported that apple sample contaminated with diazinon pesticides at a concentration level of 80 µg/kg.

Among the 4 samples analyzed apple was the commodity contain three class of pesticide residue. Based on the information gathered from retailers most of apple imported from different countries. Our finding revealed that apple sample was contaminated with pesticide residue. To date, imported fruits and vegetables are not monitored for the presence of pesticide residues. Therefore, imported fruits and vegetables may be additional source of contamination.

4.3 Multiple pesticide residues

Two or more pesticides residues were detected in 80 % of analyzed samples, 25% of the samples contained two pesticide residues and another 25% of the samples contained four pesticides residues respectively, while 20% of the samples contained three pesticide residues. Residues of five pesticides were detected in 10% of the samples as shows in Figure 4.8.

Of the 21 pesticides studied, 8 were detected in the fresh lettuce samples as indicated in figure 4.9. More than two residues were detected in lettuce sample; 8 samples contaminated with more than 4 different active ingredients; with a frequency of occurrence as follows: lindane, heptachlor and endosulfane.

Figure 4.8 illustrates the presences of two or more residues were detected in strawberry samples; 2 samples contained 4 different active ingredients; with a frequency of occurrence as follows: cypermethrin, chloridane and endosulfane. 70% of apple samples contained two or more pesticide residues; 4 samples contained two different pesticides residues; 2 samples with three different pesticides and one sample contaminated with 4 different pesticides. In the case of tomato, 3 samples contained two different pesticides residues; 3 samples with three different pesticides; 2 samples with 4 and one sample contaminated with 5 different pesticides.

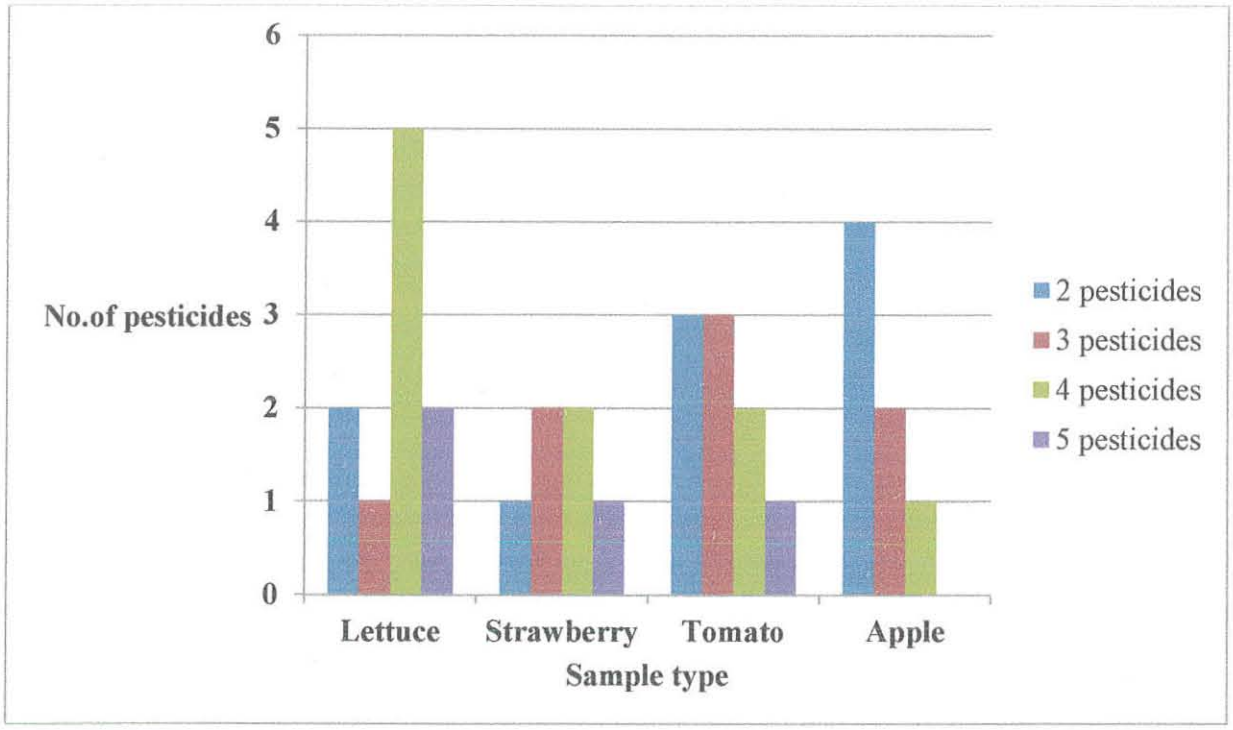


Figure 4. 8 Samples contaminated with more than two pesticides

Figure 4.9 illustrates the co-occurrences of pesticide residues of which the 10 pesticides detected out of the total of 21 investigated pesticides. The compounds most frequently detected among the 4 samples analyzed were lindane, heptachlor, endosulfane DDT and cypermethrin.

Generally, the high frequent of organochlorine and synthetic pyrethroids in the fruits and vegetables were observed as compared to organophosphate. The frequency of pesticide residues detected in the samples can be arranged in the following descending order: lettuce > tomato >strawberry > apple.

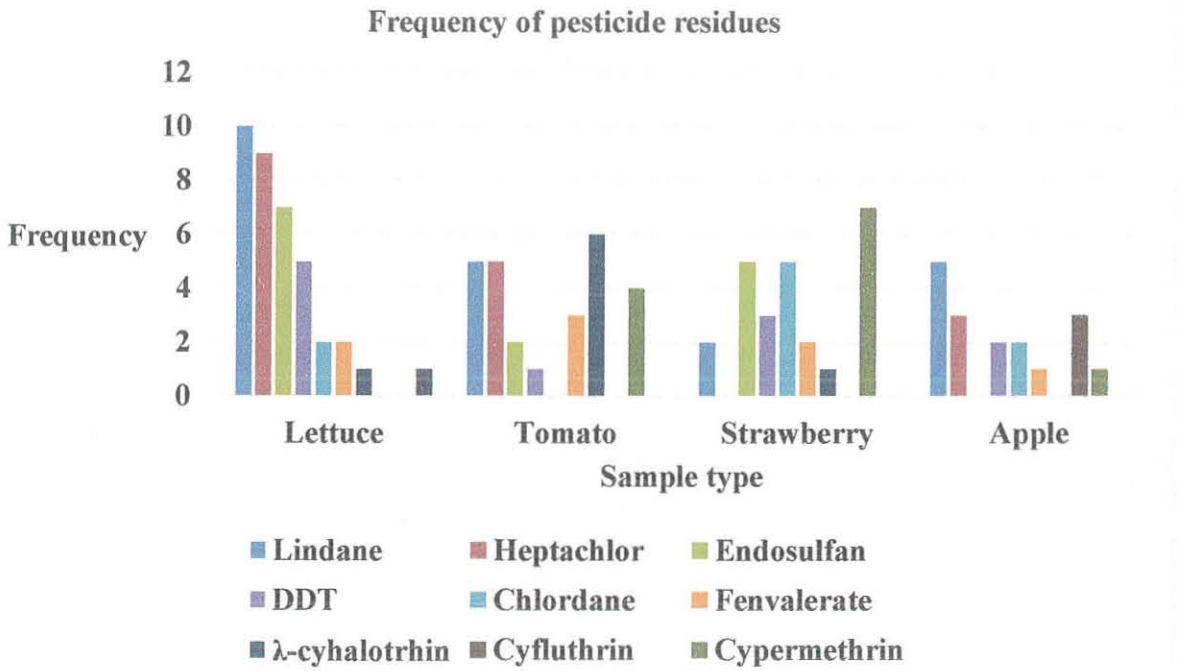


Figure 4.9 Most frequently detected pesticides

The above information indicates the occurrence of multiple - pesticide residues in the fruit and vegetable samples are common. Previous studies indicated that, it was common to find more than two pesticide residues in fruits and vegetable samples. Results from monitoring program found that 34% of the total samples from Spain (Blasco et al., 2006), 14.7% from China (Chen et al., 2011) and 47.8% from Brazil (Jardim & Caldas, 2012) contaminated with at least two pesticides. Therefore, to control fruit and vegetable from different organisms various pesticides are widely used, which is the main path leading to the presence of multiple residents. This suggests that the pesticide application frequency and pre-harvested interval must be controlled. According to the current EU legislation, the presence of multiple residues in one sample is not a reason for considering a sample as not compliant with the MRL legislation, as long as the individual residues do not exceed their respective MRLs (Mozzaquatro et al., 2019).

4.4 Maximum Residue Limits (MRLs) Compliance

The level of pesticide residues in 40 fruit and vegetable samples were determined. As shows in figure 4.10 pesticide residues were not detected in 5 samples (12.5%), while 35 samples (87.5%) contaminated with detectable amount of pesticide residues. Out of the 35 samples, 8 (20 %) contaminated with lindane, heptachlor, chlordane, diazinon and fenvalerate residue above the MRLs, whereas 27 samples (67.5 %) contained pesticide residue at or below the MRLs established by Codex and EU regulation. The percentage of contaminated samples was high for vegetables than fruits. All of the lettuce (100%) samples contain a detectable amount of pesticide residues. At least one type of pesticide was detected in studied lettuce samples. 90% of the tomatoes samples were contained the detectable amount and the residue levels were all below the MRLs set by Codex and EU regulation. All of the fruit samples had an average value of 80% samples contained detectable amount of pesticide residue: strawberry (70%) and apples (90%). Lettuce (60%), strawberry (10%) and apple (10%) recorded the highest number of samples that exceeded the MRLs (figure 4.10).

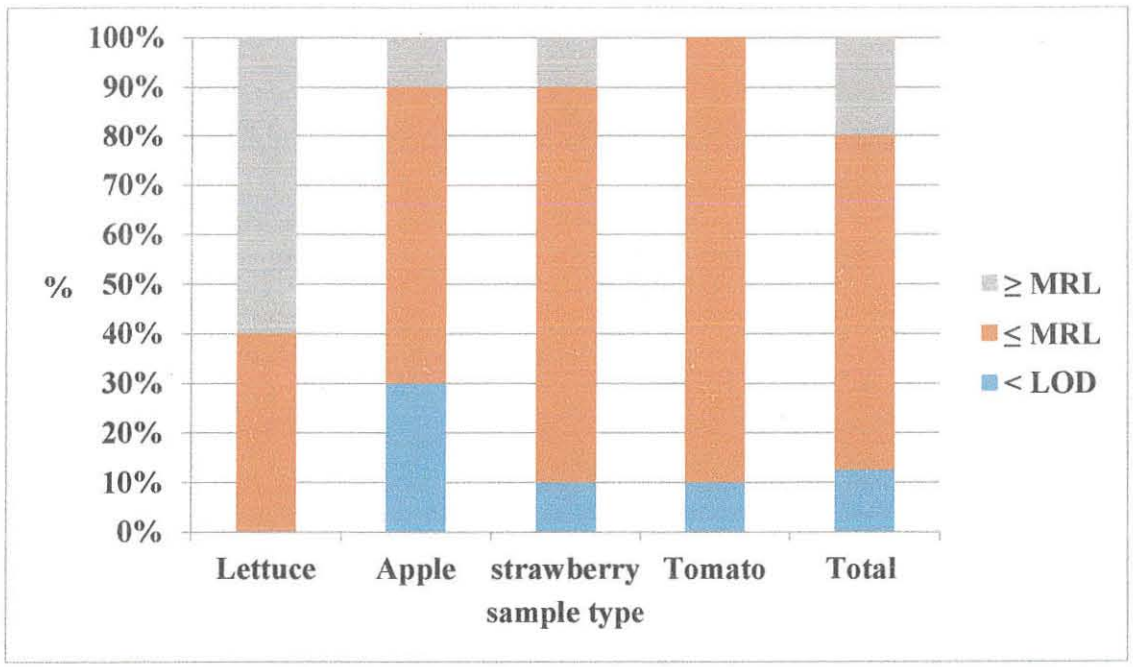


Figure 4. 10 Percentage of samples in compliance or noncompliance with the MRLs established by EU or Codex Alimentarius.

Of the 21 pesticides (including metabolites) studied, 10 pesticides were detected in the analyzed fruit and vegetable samples (Figure 4.11).

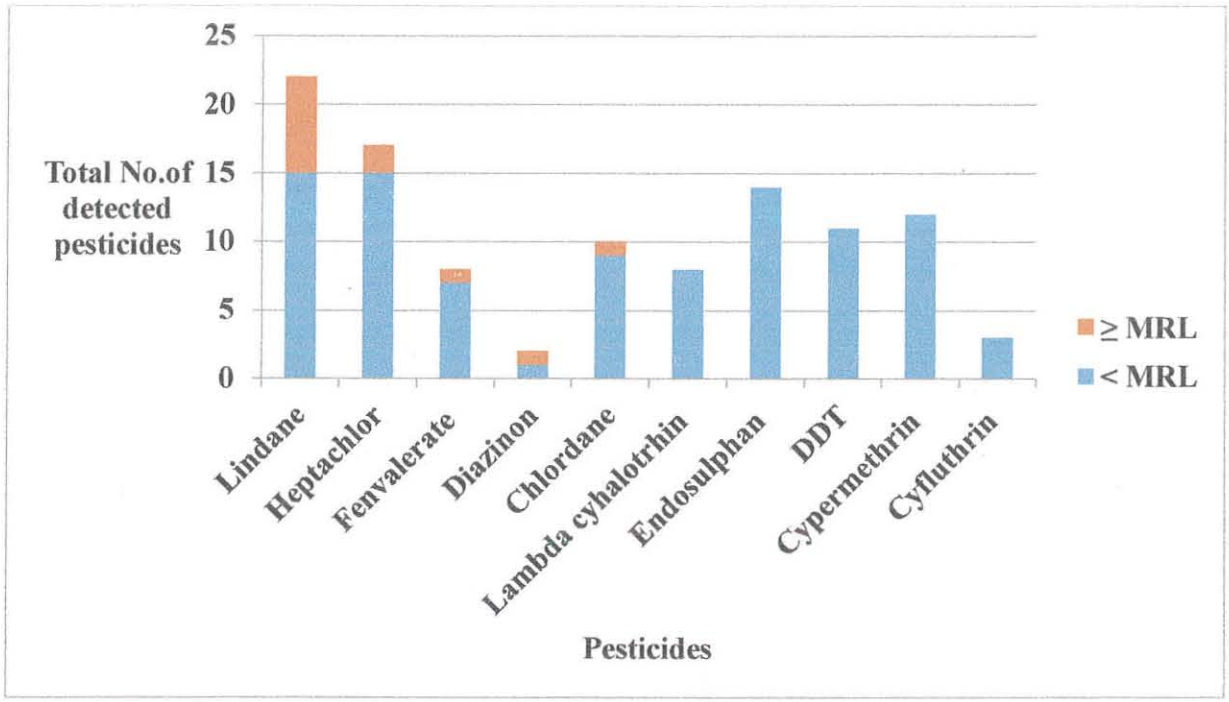


Figure 4. 11 Total detection of pesticides and compliance with the maximum residue limits.

The most common pesticides detected were lindane (22 samples), heptachlor (17 samples), endosulfan (14 samples), cypermethrin (12 samples), DDT (11 samples), chlordane (10 samples), fenvalerate and lambda-cyhalothrin (8 samples).

Residues of lindane exceeding the MRLs were detected in six samples. Residues of heptachlor that exceeded the MRLs were also found in two samples. Chlordane exceeded the MRLs in one sample. MRL exceedance was observed for fenvalerate and diazinon in one sample simultaneously. Out of the total samples analyzed, organophosphates (chlorpyrifos and malathion), organochlorines (aldrin, dieldrin and endrin) and synthetic pyrthriod (delatamethrin) were not detected in any of the samples. Residues of heptachlor, chlordane, DDT, cyfluthrin and cypermethrin were also found in apple samples but were below the MRL.

This study shows the evidence of the presence of pesticide residues in lettuce, tomato, strawberry and apple in Ethiopia. 20% of the samples analyzed contaminated with pesticide residue above MRL. Higher levels of pesticides were reported in similar studies in developing countries. Dogheim et al. (2004) reported that eight fresh produce markets in Egypt, 23.9% contained

detectable pesticides. Mutengwe et al. (2016) indicated that 36.2% of the samples from seven fresh produce markets in Mauritius. Almost double of these figures (43.5%) were reported in Ghana, samples from six fresh produce markets (Bempah et al., 2012).

Based on the data obtained this study revealed that lettuce, strawberry and apple samples analyzed contaminates with residues of the monitored organochlorines, organophosphate and synthetics pyethriod above the acceptable maximum residue limits. The presence of organophosphate and Synthetic pyrethroids in the fruits and vegetables indicated that modern pesticides used by farmers may be misused or misapplied or not controlled and cause contamination.

Generally, the high frequent and high levels of organochlorine in the fruits and vegetables analyzed are an indication that either there banned pesticides were used by farmers through smuggling or due to their persistence nature in the environment. Negatu et al. (2016) reported that there is an illegal use of restricted and banned pesticides by some farmers in Ethiopian. In addition, persistence and dangerous pesticides like DDT, aldrin and heptachlor were dumped or stored in different sites in Ethiopia (Mekonen et al., 2014) from where it could enter the agricultural soils and water systems and possibly find its way into crops.

It should be understood that MRLs are not safety limits, a food residue can have higher level than MRL but can still be safe for consumption (Valavanidis, 2016). Safety limits are assessed in comparison with acceptable daily intake (ADI) for short term exposure or acute reference dose (ARfD) (Neme & Satheesh, 2016) and are therefore exposure of consumer to pesticide residue through the consumption of fruit and vegetable should be assessed to draw decisive conclusion with respect to exposure of consumers to pesticide.

4.5 Washing experiment

Among the household processes, washing is the most common practice in processing of fruits and vegetables. Researchers have examined the effect of washing process to remove pesticides in fruits and vegetables (Chavarri et al., 2005; Satpathy et al., 2012; Soliman, 2001). In present study, washing was found comparatively less effective in reducing the residues of SP pesticides than that of OCP as it depends on a number of factors like location of residues, age of residues, water solubility and temperature and type of washing.

Paired t-test analyses were performed to compare between residuals in washed and unwashed fruit and vegetables samples. Paired t-test revealed that in most of the comparisons between the washed and unwashed samples, no-significant differences were observed ($P > 0.05$). Refer to (Appendix C) for the t-test analysis data. It means that most of the pesticides remain on the skin of fruits and vegetables after washing with water such as tomatoes (Bajwa & Sandhu, 2014).The results of this study are comparable with those obtained by washing elsewhere; as washing only removes loose surface residues and major portions of polar compounds only. For example, Al-Shamary et al. (2016) reported that the pesticide residues that are on the surface of vegetables require two to three times washings. Soliman (2001), noted that reduction of organochlorine pesticides residues were more efficient by washing the vegetables with acetic acid or sodium chloride solutions compared to washing with tap water. This could explain why there were no differences in the presence of SP substances between washed samples by tap water and unwashed samples. Moreover, Rasmusssen et al. (2003) indicated that the juicing and peeling of apples significantly reduced all pesticide residues, while none of the pesticide residues were significantly reduced by washing.

The result obtained in this study is inconsistent with some of the reports in the literature. For example the result reported by Kumari (2008) and Malik et al. (1998) showed that washing reduced 10-30% for fenvalerate residues in tomatoes. Jain et al. (1979) reported that washing reduced the SP residues by 62%,while Chauhan et al. (2012) showed rinsing of various vegetables reduce the SP by 74-84%. (Krol et al., 2000) concluded that rinsing effectively reduces SP residues in vegetables.

Moreover, the efficiency of the washing treatments on pesticide dislodging depends on the washing solution, the chemical properties of the pesticide, the surface area, the nature of the food, the length of time the pesticide is in contact with the food, and the formulation and application method of the pesticide. Usually, the pesticide is lodged in the outer wax-like layers and then moves to the inside, making washing and removal of the pesticides less effective (Neme & Satheesh, 2016)

Though, it seems that the effects of washing samples with tap water differ in organochlorine residues based on the type of vegetables and fruits. Statistically significant differences ($P \leq 0.05$) in presence of some organochlorine compounds between washed and unwashed samples were

noted in lettuces and strawberries. Endosulfan compound in lettuce and strawberry showed significant difference between the washed and unwashed samples (P = 0.0465) and (P = 0.0168), respectively. Meanwhile, lindane in lettuce showed significant difference between the washed and unwashed samples (P = 0.0152). Refer to (Appendix C) for the t-test analysis data.

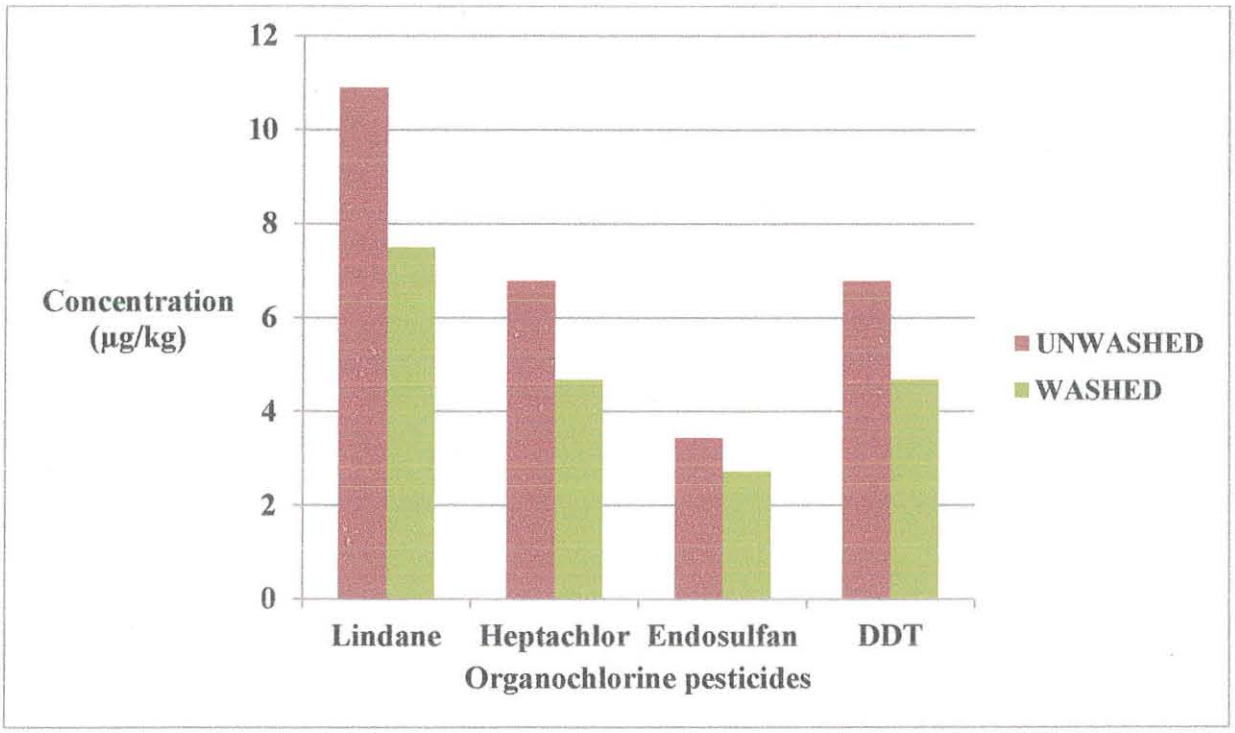


Figure 4. 12 Comparison of unwashed and washed lettuce sample.

Figure 4.12 illustrate that the pesticide residue level in lettuce especially organochlorine shows reduction after washing with tap water for 2 minutes. Therefore washing reduced residual burden of lindane in lettuce as the residues fell below maximum residue limits.

4.6 Knowledge, attitude and practice (KAP) to wards pesticide residue

4.6.1 General information

A total of fifty five consumers participated in the study. The participants were 58% male and 42% female. The majority of the respondents were in the range of 15-30 (62%) and 31-40 years old (22 %), while 9% and 7% of them were in the range of 41-50 and younger than 51 years old, respectively. The study of education level shows that 96% of them had been educated, 11 % had

been educated up to primary school, and 22% had been educated up to secondary school. In addition, all of the respondents consumed at least one type of fruits and vegetables.

4.6.2 KAP towards pesticide residue

The score level of KAP was classified into 3 levels as follows: Bloom's Theory (Bloom, 1956):Score: Less than 60% = Low levels; 60-80% = Moderate levels and 81-100% = High level. The distribution of the knowledge of the respondents shows that 15 % of respondents had low and high knowledge level, while 60% of them had moderate knowledge level (Table 4.7).

Table 4. 6 Consumers' knowledge on pesticide residue

Knowledge towards pesticide residue	**Respond% (n=55)**			
Statement	**1**	**2**	**3**	**Level**
1. Do you know about pesticides?	82	18	na	High level
2. Do you know that pesticides affect human health?	71	9	20	Moderate level
3. Do you know that pesticides are used in fruits and vegetables production?	69	6	25	Moderate level
4. Do you know that a person can be affected with pesticide through food?	65	6	29	Moderate level
5. Do you know the responsible body for regulating pesticide use in the country?	15	85	na	Low level

High level (81% - 100%); Moderate level (60% – 80%); Low level (Less than 60%)

Note 1=yes; 2=no; 3=I don't know; na =not applicable

In this study, 71% of the participants were aware that pesticides exert adverse effects on human health. This is in agreement with the result of Recena et al. (2006), who found that over 65% of the participants considered that use of pesticides, could adversely affect their health.

More than 75 % of consumers involved in the study believed that the presence of pesticide residues is highly unsafe for health. 75% of the consumers reported about thorough washing of vegetables before cooking, but 46 % consumers felt that washing removes pesticide residues only to a limited extent (Suresh et al., 2015)

The distribution of consumers' attitudes level was shown in Table 4.8. About 67% of them had low attitude, 33% of them had moderate attitude, while none of the consumer had high attitude.

The distribution of respondents' practice shows that, there was moderate level of practice (table 4.9).

Table 4. 7 Consumers' attitude on pesticide residue

Attitude of consumers' towards pesticide residue	**Respond% (n=55)**				
Statement	**1**	**2**	**3**	**4**	**Level**
1. Do you think fresh fruits and vegetables contaminated with pesticide?	53	18	29	na	Low level
2. Do you believe that washing will remove pesticide residues from fruit and vegetable completely?	35	22	7	36	Moderate level
3. Do you think regulating pesticide is effective in the country?	36	55	9	na	Low level

High level (81% - 100%); Moderate level (60% – 80%); Low level (Less than 60%)

Note 1=yes; 2=no; 3=I don't know; 4=slightly; na= not applicable

Table 4. 8 Consumers' practice on pesticide residue.

Practice of consumers' towards pesticide residue	**Respond% (n=55)**			
Statement	**1**	**2**	**3**	**Level**
1. Do you have practice of washing fruits and vegetables before consuming?	69	0	31	Moderate level
2. Do you care about the source of fruits and vegetables?	65	35	na	Moderate level

High level (81% - 100%); Moderate level (60% – 80%); Low level (Less than 60%)

Note 1=yes; 2=no; 3=sometimes; an= not applicable

In general from the KAP survey consumers' have moderate level of knowledge, attitude and practice of pesticide residue in fruit and vegetables.

Chapter five

5 Conclusions and recommendations

5.1 Conclusions

The results of this study indicate that commercially available lettuce ,tomato, strawberry and apple samples in Addis Ababa markets contains one or more type of pesticides residues. Among OC and SP were the most common ones. Lindane (α-BHC and δ-BHC), DDT and fenvalerate were the most frequently detected pesticides.

Out of the thirty-five positive samples, thirty-two samples (80%) contaminated with multiple pesticide residues. The diverse and multi-detected above threshold pesticide residues limits found here are also a cause of concern.

Seven organochlorine pesticides residues were detected of which six are among the banned pesticides in Ethiopia. Lindane was the most common OCP detected while lettuce was the most frequently contaminated sample.

The concentration of lindane and heptachlor in lettuce samples, chlordane in strawberry sample and fenvalerate and diazinion in apple sample exceeded the maximum residue limits (MRLs) adopted by the FAO/WHO Codex Alimentarius Commission and EU commission. All pesticides detected in tomato samples were below MRLs.

The differences in residual concentrations of pesticides could be due to different agricultural practices adopted by farmers and also accessibility of the pesticides. The results also underscore the need for regular monitoring of large samples to determine the pesticide residues for locally produced as well as imported samples.

Our washing results demonstrated that washing of fruits and vegetable with running tap water can remove the pesticide residues but not all of them.

In general from the KAP survey consumers' have a moderate level of knowledge, attitude and practice of pesticide residue in fruit and vegetables.

5.2 Recommendations

Based on the results of the present study the following recommendations are made:-

- A more comprehensive monitoring program, incorporating more locations and following the growing periods of fruits and vegetables, is required to draw decisive conclusions with respect to sources of contaminations.

- The pesticide residues which were found beyond limit clearly indicate the need for the establishment of a national monitoring program oriented towards locally produced and imported fruits and vegetables. In doing so it would be possible to control the residue limits for safe consumption.

- Policymakers and other stakeholders alike put in place stringent monitoring, regulating and enforcing frameworks on existing laws for good pesticide use practices by farmers.

- There is a need to conduct a dietary exposure for pesticide residue through the consumption of fruits and vegetable.

- Consumers are strongly recommended to wash fruits and vegetable with water before consuming is important in order to reduce residual burden.

- Farmer's education on safe pesticide use should be intensified to limit the levels of pesticides residues and public awareness should be initiated about the pollution status of fruits and vegetables.

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Appendices

Appendix-A Linearity of matrix match calibration curve

Table A 1 Linearity of matrix match calibration curve.

Pesticides	Apple		Lettuce		Strawberry		Tomato	
	Linear Equation	R^2	Linear Equation	R2	Linear Equation	R2	Linear Equation	R2
α-BHC	Area = 2095426.3*Amt - 2803.52	0.9986	Area = 1866982.52*Amt +9467.02	0.9992	Area = 1820690.27*Amt +3755.45	0.9979	Area = 2899181.77*Amt -7721.841	0.9986
Diazinon	Area = 97544.1565*Amt +7029.9183	0.9932	Area = 94839.516*Amt +1013.5869	0.9999	Area = 100401.19*Amt +640.48804	0.9995	Area = 675525.903*Amt -1850.8138	0.9945
δ- BHC	Area = 1052823.33*Amt +678.05781	0.9991	Area = 906531.282*Amt +904.57326	0.9968	Area = 2308683.11*Amt +1290.8167	0.9998	Area = 2989107.52*Amt -17417.193	0.9947
Heptachlor	Area = 2351275.72*Amt -355.75546	0.9989	Area = 1826227.41*Amt -2045.592	0.9995	Area = 2137598.57*Amt +917.55106	0.9999	Area = 3420716.43*Amt -6422.2925	0.99881
Aldrin	Area = 2551374.28*Amt +1642.6191	0.9987	Area = 2263587.2*Amt -1763.8405	0.9995	Area = 2209772.98*Amt -951.39258	0.9999	Area = 3467888.34*Amt -12712.998	0.9986
Chlorpyrifos	Area = 1019173.35*Amt -8655.0975	0.9995	Area = 1012803.78*Amt -16524.957	0.9990	Area = 897098.853*Amt -1802.6435	0.9983	Area = 7166257.44*Amt -36594.734	0.99697
Malathion	Area = 199302.876*Amt -1468.1618	0.9965	Area = 203021.245*Amt -2520.5006	0.9988	Area = 250447.865*Amt +2265.1913	0.9988	Area = 1689150.55*Amt -6671.5388	0.99667
γ- Chlordane	Area = 2184595.06*Amt -390.68044	0.9996	Area = 2043487.02*Amt +312.22273	0.9947	Area = 2239540.28*Amt -1005.2757	0.9977	Area = 3286212.85*Amt -13628.15	0.99645
α-Endosulphan	Area = 1940929.79*Amt -3995.2926	0.9995	Area = 1869522.8*Amt -2307.148	0.9996	Area = 2080530.11*Amt +119.19122	0.9987	Area = 2898682.96*Amt -9595.0596	0.99691
p,p DDE	Area = 2347186.06*Amt -3350.3022	0.9987	Area = 2085517.5*Amt -4961.3001	0.9994	Area = 2316306.97*Amt -3313.0432	0.9971	Area = 3260764.82*Amt -14216.247	0.9973
Dieldrin	Area = 2331933.42*Amt -6153.5189	0.9994	Area = 2357306.37*Amt -7036.0074	0.9992	Area = 2612578.81*Amt +283.19663	0.9988	Area = 3589552.45*Amt -14386.237	0.99686
Endrin	Area = 1958400.73*Amt -5071.1684	0.9992	Area = 1809994.71*Amt -3856.3101	0.9996	Area = 2131482.65*Amt +2202.1851	0.9991	Area = 2473291.25*Amt -10065.26	0.99711
p,p DDD	Area = 1992041.16*Amt -3814.9822	0.9996	Area = 2210295.57*Amt -6190.387	0.9990	Area = 2082237.31*Amt -233.43099	0.9977	Area = 2841350.53*Amt -14538.648	0.99652
p,p DDT	Area = 1294260.27*Amt -5625.7834	0.9976	Area = 926850.39*Amt -2430.7571	0.9989	Area = 1982141.51*Amt -1810.0913	0.9975	Area = 2370463.49*Amt -12533.832	0.99633
Endosulphan sulphat	Area = 872989.461*Amt -1711.8386	0.9987	Area = 850834.376*Amt -487.15685	0.9990	Area = 1649139.38*Amt +2719.8629	0.9985	Area = 2192969.28*Amt -10464.164	0.99694
Metoxychlor	Area = 740816.656*Amt -1551.4457	0.9993	Area = 510347.991*Amt -1679.4336	0.9982	Area = 961302.99*Amt +395.72179	0.9989	Area = 1159918.35*Amt -5192.3894	0.99716
λ-cyhalotrhin l	Area = 159796.761*Amt -399.74233	0.9992	Area = 433557.912*Amt -10100.293	0.9971	Area = 37973.2007*Amt -217.97649	0.9984	Area = 567063.036*Amt -1840.2061	0.99507

λ- cyhalothrin 2	Area = 1540552.71*Amt -48599.662	0.99196	Area = 835841.725*Amt -7386.886	0.9958	Area = 1759575.08*Amt -5516.9505	0.9977	Area = 11255280.9*Amt -16432.162	0.9965
Cyfluthrin 1	Area = 280163.02*Amt - 5060.4893	0.9989	Area = 187465.19*Amt -2161.259	0.9964	Area = 347785.458*Amt -1549.425	0.9978	Area = 2534703.3*Amt - 13159.654	0.99667
Cyfluthrin 2	Area = 348719.699*Amt -7693.3841	0.99808	Area = 206716.316*Amt -366.76661	0.9981	Area = 470805.187*Amt -1846.6676	0.9988	Area = 3496534.03*Amt -22171.477	0.99554
Cyfluthrin 3	Area = 218973.629*Amt -996.449	0.9964	Area = 172550.561*Amt -2328.472	0.9968	Area = 272561.822*Amt -782.71332	0.9990	Area = 2109801.22*Amt -11665.817	0.99608
Cyfluthrin 4	Area = 252856.365*Amt -3614.1898	0.99927	Area = 177570.867*Amt -2007.0801	0.9958	Area = 308085.335*Amt -251.42105	0.9988	Area = 2361662.99*Amt -14893.068	0.99523
Cypermethrin 1	Area = 170266.904*Amt -2497.4727	0.99572	Area = 312055.236*Amt -5850.5039	0.9978	Area = 29774.1863*Amt -135.34199	0.9975	Area = 413343.712*Amt -822.85506	0.99208
Cypermethrin 2	Area = 803399.95*Amt - 20060.129	0.99638	Area = 325265.286*Amt +2792.1727	0.9972	Area = 1138821.09*Amt -3801.8334	0.9988	Area = 7113922.02*Amt -23259.281	0.99462
Fenvalerate 1	Area = 701504.053*Amt -18424.048	0.9965	Area = 352302.026*Amt -6364.9249	0.9966	Area = 965409.68*Amt - 3621.7784	0.9991	Area = 7266962.41*Amt -45785.413	0.9958
Fenvalerate 2	Area = 323370.142*Amt -2371.6027	0.99948	Area = 248234.61*Amt -3127.481	0.9967	Area = 262055.297*Amt -522.33598	0.9993	Area = 2309061.18*Amt -13917.712	0.99394
Deltamethrin 1	Area = 272684.143*Amt -408.93712	0.9963	Area = 235288.527*Amt -3932.4434	0.9967	Area = 42282.6172*Amt -83.060918	0.99987	Area = 594310.502*Amt -1569.9792	0.9957
Deltamethrin 2	Area = 702251.407*Amt -16427.342	0.99672	Area = 220506.322*Amt +2178.4332	0.9948	Area = 1209507.79*Amt -3615.3906	0.99938	Area = 8462030.87*Amt -53671.339	0.9956

R^2 coefficient of correlation

Appendix-B GC-MS ion chromatogram

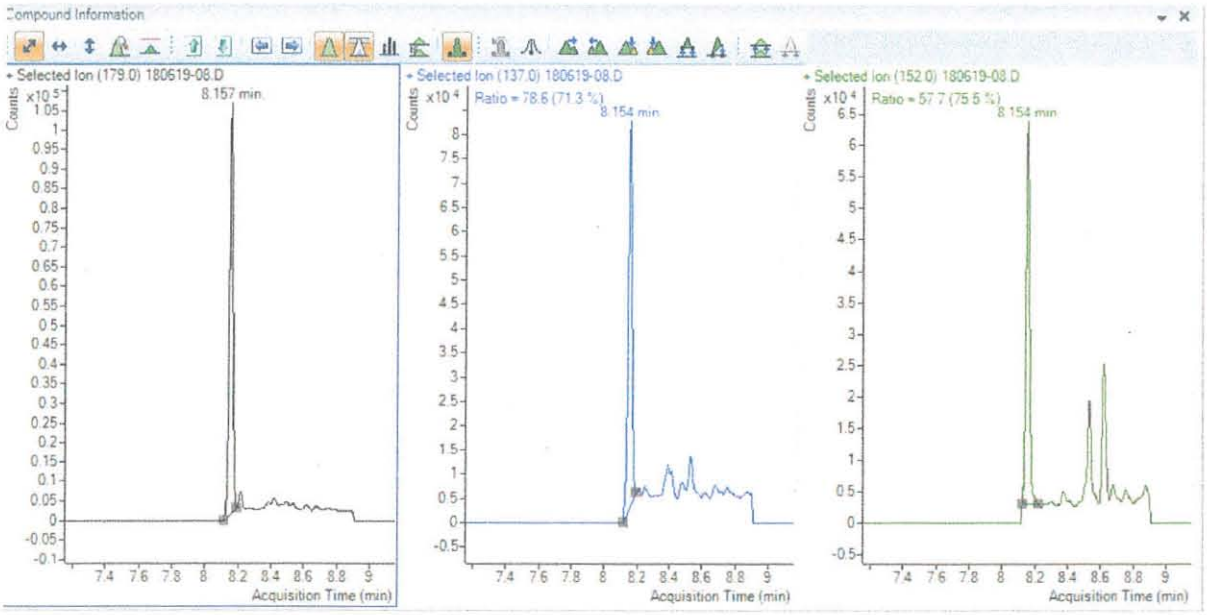

Figure B. 1 Ion chromatogram of apple sample contaminated with diazinon pesticide residue.

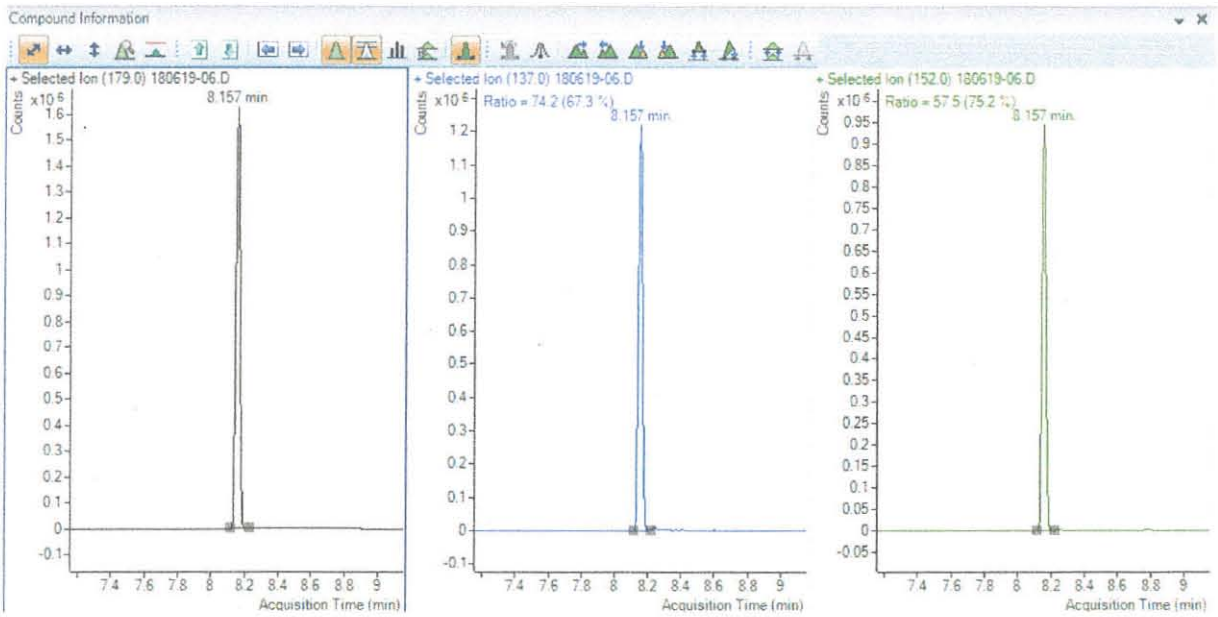

Figure B. 2 Ion chromatogram of diazinon pesticide standard.

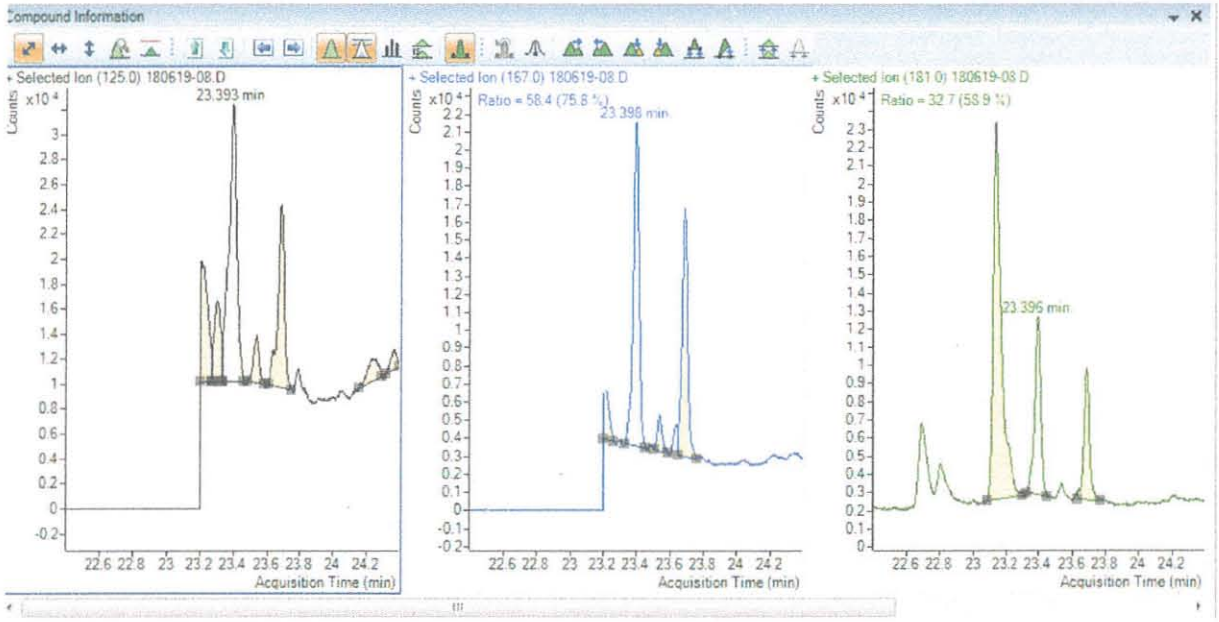

Figure B. 3 Ion chromatogram of apple sample contaminated with fenvalerate pesticide residue.

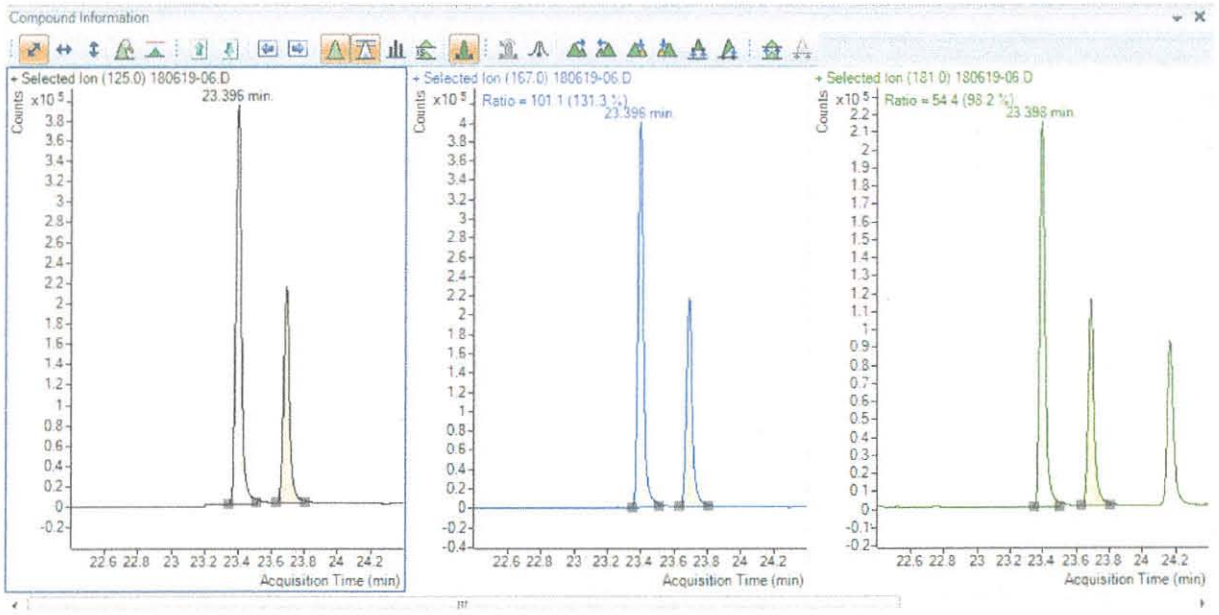

Figure B. 4 Ion chromatogram of fenvalerate pesticide standard.

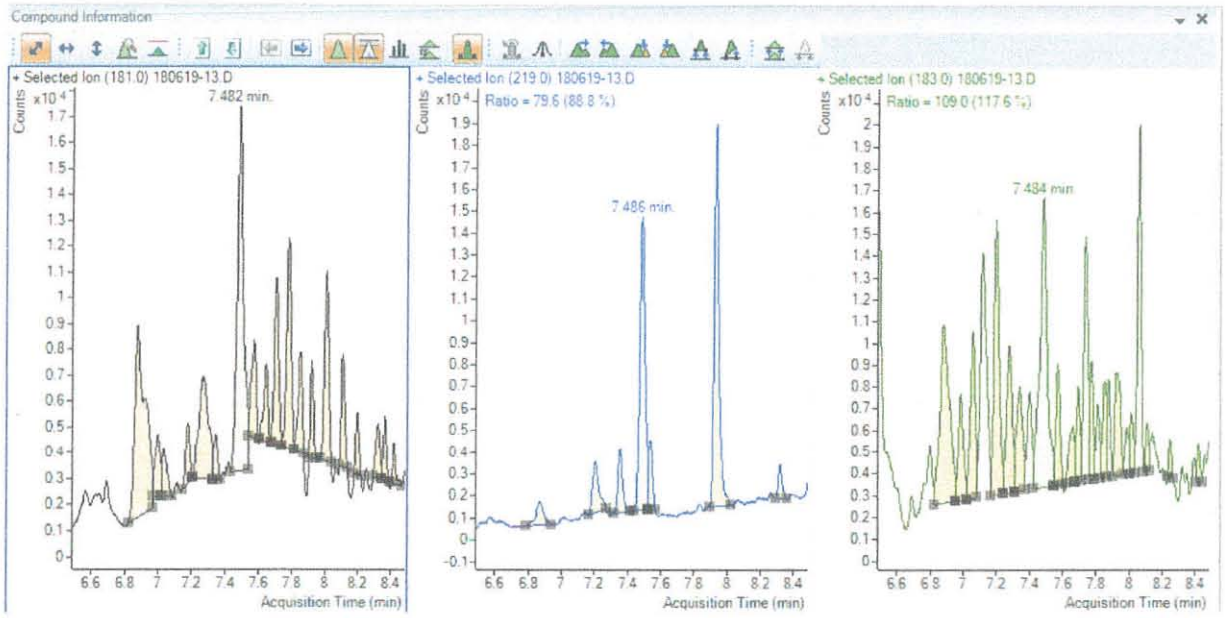

Figure B. 5 Ion chromatogram of lettuce sample contaminated with α-BHC pesticide residue.

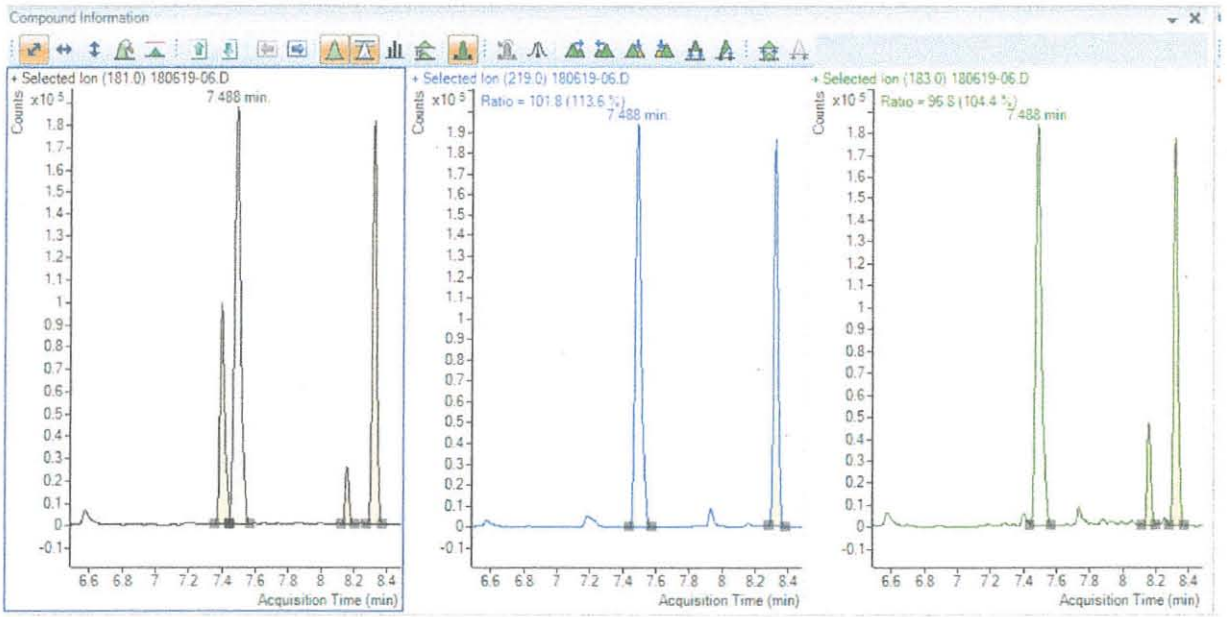

Figure B. 6 Ion chromatogram of α-BHC pesticide standard.

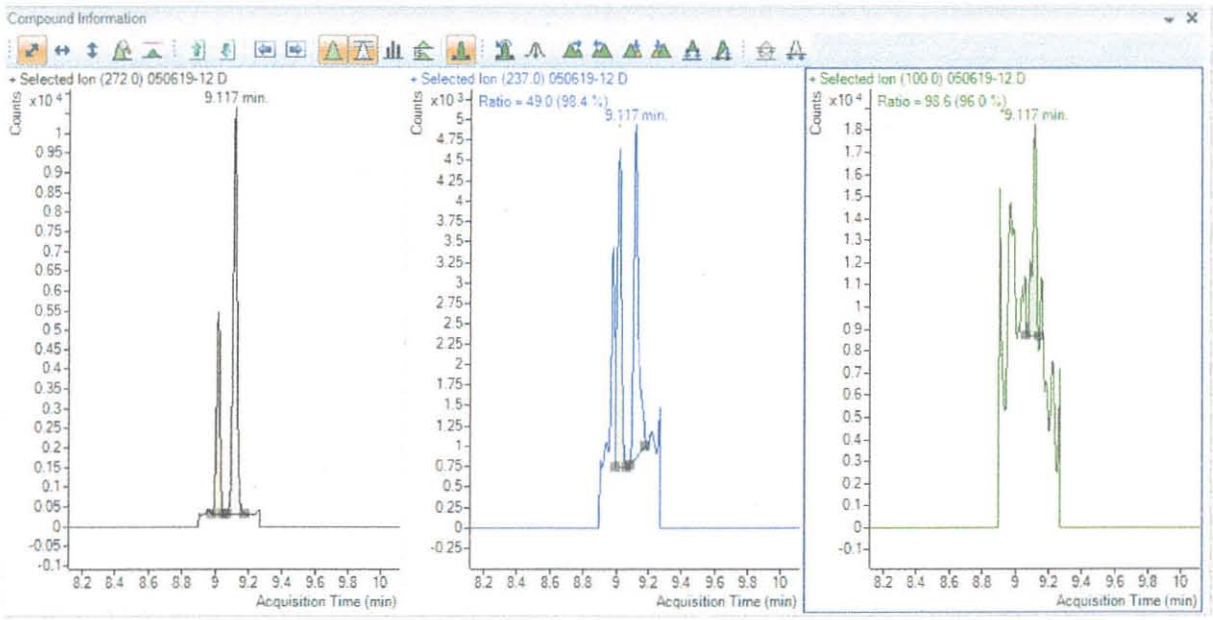

Figure B. 7 Ion chromatogram of lettuce sample contaminated with Heptachlor pesticide residue.

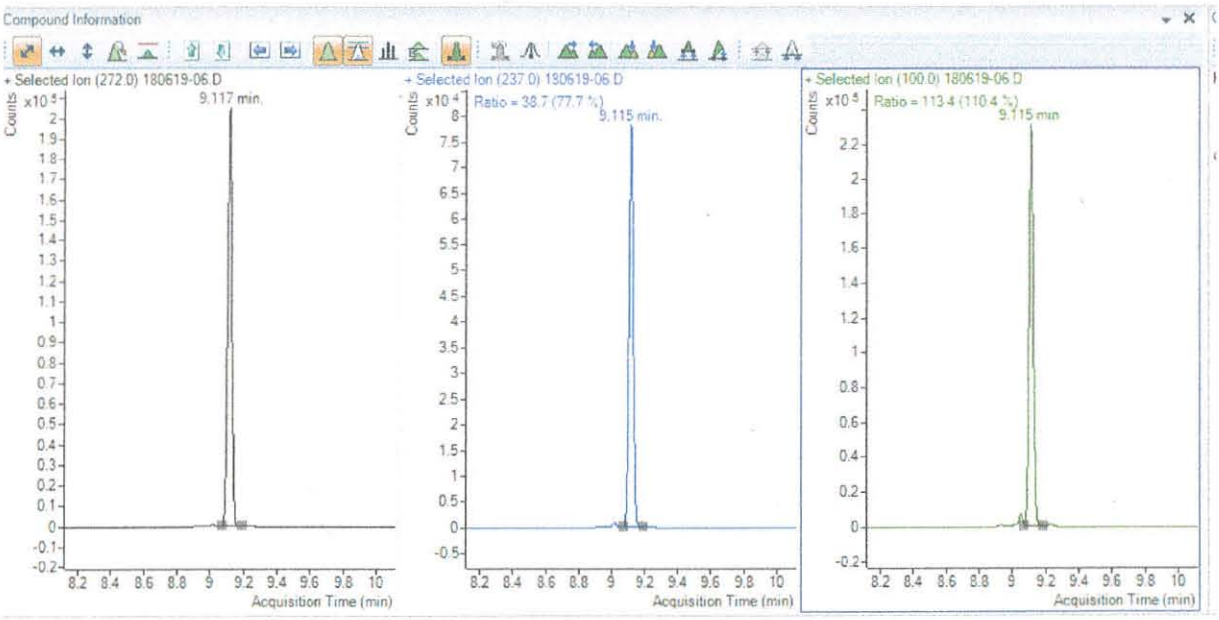

Figure B. 8 Ion chromatogram of heptachlor pesticide standard.

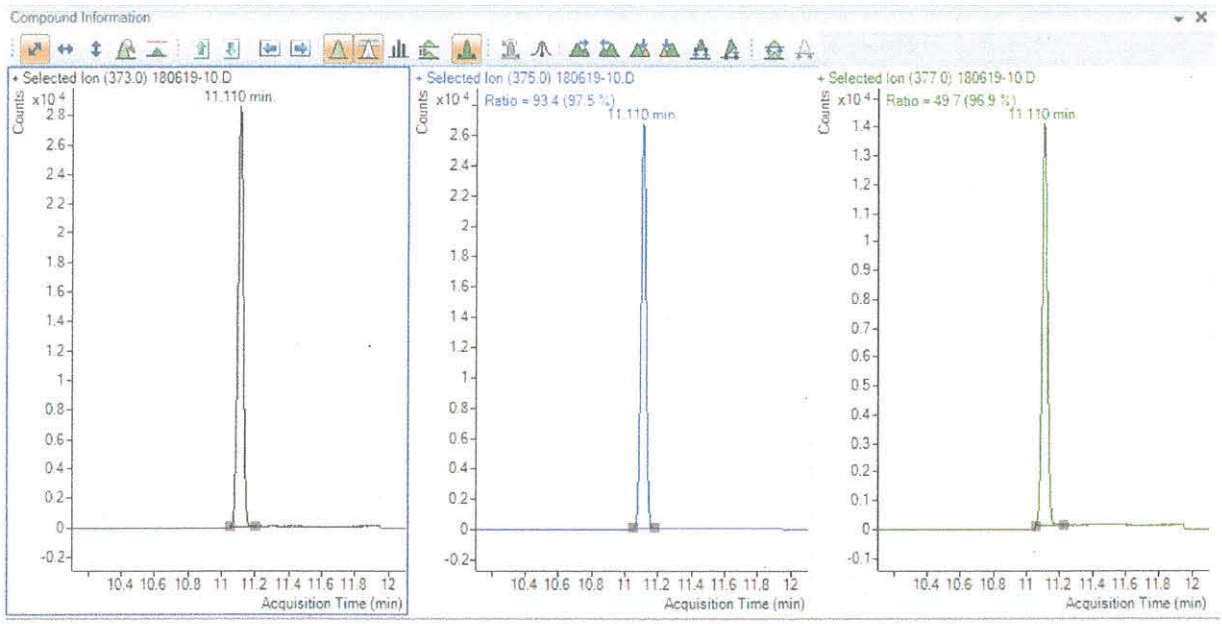

Figure B. 9 Ion chromatogram of strawberry sample contaminated with γ-chlordane pesticide residue.

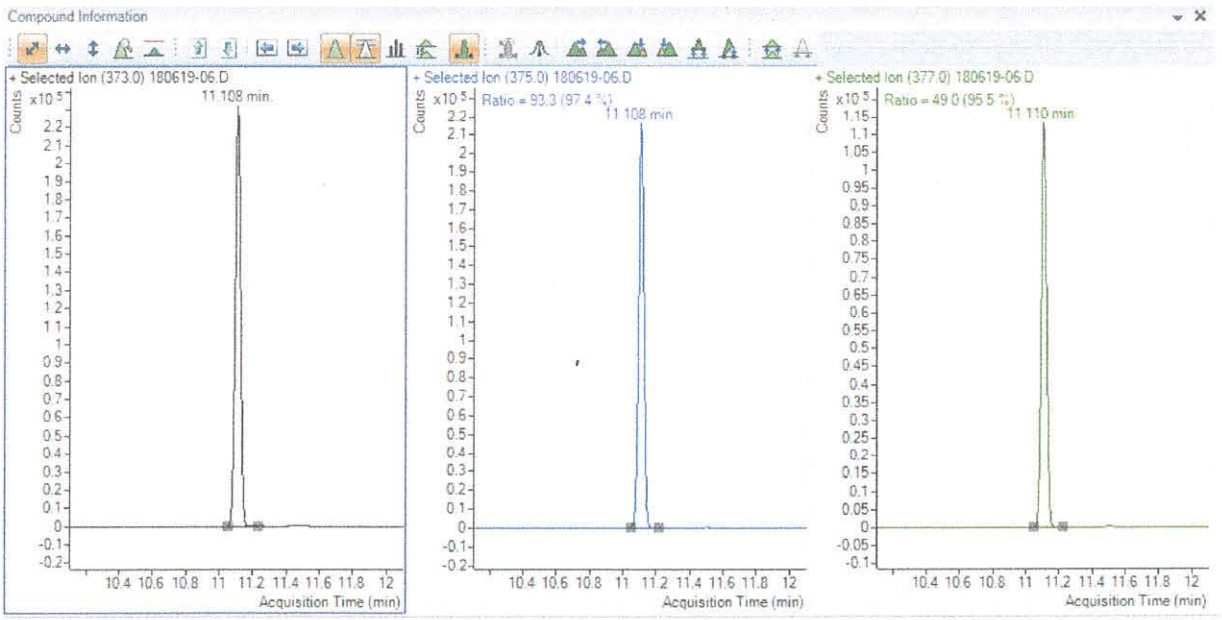

Figure B. 10 Ion chromatogram of γ-chlordane pesticide Standard.

Appendix-C Paired t-taste for washed and unwashed sample

Table C. 1 Lettuce paired t-taste

Lettuce Lindane (µg/kg)		Lettuce DDT (µg/kg)		Lettuce Endosulphan (µg/kg)		Lettuce Heptachlor (µg/kg)	
unwash	wash	unwash	wash	unwash	wash	unwash	wash
11	5	3	3	3	2	5	2
19	9	3	3	4	3	4	4
11	10	4	3	3	3	4	4
7	3	4	4	6	4	7	3
14	12	3	4	3	3	4	1
6	6			2	2	11	4
10	10			3	2	4	1
8	3					12	3
9	10					10	20
14	7						

t-Test: Paired Two Sample for t-Test: Paired Two Sample for t-Test: Paired Two Sample for t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*		*Variable 1*	*Variable 2*		*Variable 1*	*Variable 2*		*Variable 1*	*Variable 2*
Mean	10.9	7.5	Mean	3.4	3.4	Mean	3.42857	2.71429	Mean	6.77778	4.66667
Variance	15.2111	10.0556	Variance	0.3	0.3	Variance	1.61905	0.57143	Variance	11.1944	34.5
Observati	10	10	Observati	5	5	Observati	7	7	Observati	9	9
Pearson C	0.49862		Pearson C	0.16667		Pearson C	0.84163		Pearson C	0.42192	
Hypothesi	0		Hypothesi	0		Hypothesi	0		Hypothesi	0	
df	9		df	4		df	6		df	8	
t Stat	2.98967		t Stat	0		t Stat	2.5		t Stat	1.17382	
P(T<=t) o	0.0076		P(T<=t) o	0.5		P(T<=t) o	0.02326		P(T<=t) o	0.13711	
t Critical c	1.83311		t Critical c	2.13185		t Critical c	1.94318		t Critical c	1.85955	
P(T<=t) t	**0.01521**		**P(T<=t) t**	**1**		**P(T<=t) t**	**0.04653**		**P(T<=t) t**	**0.27422**	
t Critical t	2.26216		t Critical t	2.77645		t Critical t	2.44691		t Critical t	2.306	

Table C. 2 Apple paired t-taste

Apple Lindane (µg/kg)		Apple Cyfluthrin (µg/kg)		Apple Heptachlor (µg/kg)	
unwash	wash	unwash	wash	unwash	wash
3	3				
6.1	4.9	22	22	3.9	3.8
3.3	3.2	21	24	4.1	3.9
96.7	128.5	23	23	3.9	3.8
4	4				

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	22.62	28.72
Variance	1716.42	3111.8
Observations	5	5
Pearson Correlation	0.99987	
Hypothesized Mean	0	
df	4	
t Stat	-0.9488	
P(T<=t) one-tail	0.19822	
t Critical one-tail	2.13185	
P(T<=t) two-tail	**0.39644**	
t Critical two-tail	2.77645	

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	22	23
Variance	1	1
Observations	3	3
Pearson Correlat	-0.5	
Hypothesized M	0	
df	2	
t Stat	-1	
P(T<=t) one-tail	0.211325	
t Critical one-tai	2.919986	
P(T<=t) two-tail	**0.42265**	
t Critical two-tai	4.302653	

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	3.966667	3.83333
Variance	0.013333	0.00333
Observations	3	3
Pearson Correl	1	
Hypothesized M	0	
df	2	
t Stat	4	
P(T<=t) one-tai	0.028595	
t Critical one-ta	2.919986	
P(T<=t) two-tail	**0.057191**	
t Critical two-ta	4.302653	

Table C. 3 Tomato paired t-taste

Tomato		Tomato λ- cyhalotrhin (µg/kg)				Tomato		Tomato Heptachlor (µg/kg)	
Tomato		unwash	wash					unwash	wash
Lindane (µg/kg)		33.42	28.74	Tomato		Tomato		3.05	4.00
unwash	wash	42.37	32.26	Cypermethrin (µg/kg)		Fenvalerate (µg/kg)		3.00	2.97
3.88	3.72	35.49	53.46	unwash	wash	unwash	wash	3.51	3.15
3.80	3.90	55.97	41.33	15.60	9.69	73.17	73.44	3.42	3.79
4.16	4.23	55.88	46.18	18.61	10.24	72.55	74.02	3.74	3.63
3.92	3.88	55.21	49.13	179.41	123.74	74.80	72.97	3.02	3.15
4.21	4.08	15.60	9.69	198.57	164.59			4.21	3.60

t-Test: Paired Two Sample for M t-Test: Paired Two Sample for t-Test: Paired Two Sample for t-Test: Paired Two Sample for t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*		*Variable 1*	*Variable 2*		*Variable 1*	*Variable 2*		*Variable 1*	*Variable 2*		*Variable 1*	*Variable 2*
Mean	3.993323	3.96262	Mean	41.9908	37.2546	Mean	103.049	77.061	Mean	73.5071	73.4772	Mean	3.42332	3.46988
Variance	0.0336	0.03881	Variance	229.274	226.245	Variance	9910.93	6281.5	Variance	1.35195	0.27771	Variance	0.20119	0.1474
Observati	5	5	Observati	7	7	Observati	4	4	Observati	3	3	Observati	7	7
Pearson C	0.818346		Pearson C	0.75466		Pearson C	0.99107		Pearson C	-0.9494		Pearson C	0.26023	
Hypothesi	0		Hypothesi	0		Hypothesi	0		Hypothesi	0		Hypothesi	0	
df	4		df	6		df	3		df	2		df	6	
t Stat	0.595141		t Stat	1.18529		t Stat	2.2105		t Stat	0.03102		t Stat	-0.2421	
P(T<=t) o	0.291892		P(T<=t) o	0.14036		P(T<=t) o	0.05702		P(T<=t) o	0.48903		P(T<=t) o	0.4084	
t Critical c	2.131847		t Critical c	1.94318		t Critical c	2.35336		t Critical c	2.91999		t Critical c	1.94318	
P(T<=t) t	**0.583784**		P(T<=t) t	**0.28071**		P(T<=t) t	**0.11405**		P(T<=t) t	**0.97807**		P(T<=t) t	**0.8168**	
t Critical t	2.776445		t Critical t	2.44691		t Critical t	3.18245		t Critical t	4.30265		t Critical t	2.44691	

Table C. 4 Strawberry paired t-taste

Strawberry Chloridane(μg/kg)		Strawberry Endosulfan(μg/kg)		Strawberry DDT		Strawberry Cypermethrin (μg/kg)	
unwash	wash	unwash	wash	unwash	wash	unwash	wash
5.06	5.67	4.78	4.45	0.95	0.87	15.60	9.69
4.96	6.27	4.91	4.60	2.83	1.09	18.61	10.24
6.47	6.00	5.12	4.82	0.97	1.50	7.11	6.82
27.09	26.21	1.26	1.13			10.13	5.29
9.32	19.33	0.25	0.20			5.65	7.14
						19.14	7.12
						5.37	5.57

t-Test: Paired Two Sample for t-Test: Paired Two Sample for t-Test: Paired Two Sample for t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*		*Variable 1*	*Variable 2*		*Variable 1*	*Variable 2*		*Variable 1*	*Variable 2*
Mean	10.5789	12.6958	Mean	3.26425	3.04202	Mean	1.58383	1.15548	Mean	11.659	7.40979
Variance	88.3048	90.5047	Variance	5.38727	4.82474	Variance	1.16958	0.10275	Variance	36.4155	3.5865
Observati	5	5	Observati	5	5	Observati	3	3	Observati	7	7
Pearson C	0.88699		Pearson C	0.99997		Pearson C	-0.1739		Pearson C	0.66404	
Hypothesi	0		Hypothesi	0		Hypothesi	0		Hypothesi	0	
df	4		df	4		df	2		df	6	
t Stat	-1.0527		t Stat	3.94777		t Stat	0.62862		t Stat	2.25641	
P(T<=t) o	0.17593		P(T<=t) o	0.00842		P(T<=t) o	0.29691		P(T<=t) o	0.03243	
t Critical c	2.13185		t Critical c	2.13185		t Critical c	2.91999		t Critical c	1.94318	
P(T<=t) t	**0.35187**		**P(T<=t) t**	**0.01685**		**P(T<=t) t**	**0.59382**		**P(T<=t) t**	**0.06487**	
t Critical t	2.77645		t Critical t	2.77645		t Critical t	4.30265		t Critical t	2.44691	

Appendix-D ANOVA analysis

Table D 1 Organochlorine pesticide residue

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	47.82538	3	15.94179	1.753817	**0.178883**	2.946685
Within Groups	254.5136	28	9.089773			
Total	302.339	31				

Table D 2 Synthetic pyrethriod pesticide residue

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11881.29	3	3960.431	1.403782	**0.278113**	3.238872
Within Groups	45140.14	16	2821.259			
Total	57021.43	19				

Table D 3 Organophosphate pesticide residue

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	667.4472	3	222.4824	1	**0.441099**	4.066181
Within Groups	1779.859	8	222.4824			
Total	2447.306	11				

Appendix-E Questionnaire for fruits and vegetables consumers'

Good morning/Good afternoon.

Thank you for your willingness to take this interview with me. This is Ermias Ali a master's student of the Addis Ababa University center for food science and nutrition under taking a research "Assessment of pesticide residue level in fruit and vegetable from markets in Addis Ababa". The purpose of this interview is to take information from you on the aforementioned issue. Your honest answers to these questions will help me for a better understanding of the topic, and eventually help in designing and implementing appropriate interventions to alleviate related problems. You are assured of the confidentiality of your response.

Part I Background information

Sex:

□Male □Female

Age of respondent

□15-30 □31-40 □41-50 □ > 51

Educational background

□None □ Primary □ Secondary □College

Do you use fruits and vegetables?

□Yes □No

If no .....

Tick all types of fruits and vegetables you consume

□Strawberry □Apple □ Lettuce □ Tomato □ other than listed

Part II KAP

Consumers' knowledge towards pesticide

1. Do you know about pesticides?

☐ Yes ☐ No ☐ I don't know

2. Do you know that pesticides affect human health?

☐ Yes ☐ No ☐ I don't know

3. Do you know that pesticides are used in fruits and vegetables production?

☐ Yes ☐ No ☐ I don't know

4. Do you know that a person can be affected with pesticide through food?

☐ Yes ☐ No ☐ I don't know

5. Do you know the responsible body for regulating pesticide use in the country?

☐ Yes ☐ No ☐ I don't know

If yes.....

Consumers' attitude towards pesticide

1. Do you think fresh fruits and vegetables contain pesticide residues?

☐ Yes ☐ No ☐ I don't know

2. Do you believe that washing will remove pesticide residues completely?

☐ Yes ☐ No ☐ I don't know ☐ Slightly

3. Do you think regulating pesticide is effective in the country?

☐ Yes ☐ No ☐ I don't know

Consumers' practice towards pesticide

1. Do you have practice of washing fruits and vegetables before consuming?

☐ Yes ☐ No ☐ Sometimes

2. Do you care about the source of fruits and vegetables?

☐ Yes ☐ No

Do you have any comment or suggestion.....