ADAMA SCIENCE AND TECHNOLOGY UNIVERSITY

SCHOOL OF APPLIED NATURAL SCIENCE

M.Sc THESIS ON:

ASSESSMENT OF SELECTED PESTICIDE RESIDUE LEVELS IN SOME VEGETABLES GROWN THROUGH IRRIGATION BY SMALL SCALE FARMERS IN DUGDA DISTRICT OF OROMIA REGION, ETHIOPIA

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List Abbreviations and Acronyms

ASE Accelerated Solvent Extraction

ATJK Adami Tulu Jido Kombolch

CIA Central Investment Agency

CSA Central statically agency

ECD Electron Capture Detector

EHDA Ethiopian Horticultural Development Agency

EHNRI Ethiopian Horticultural and Nutritional Research Institute

EHPEA Ethiopian Horticulture Producers and Exporters Association

EPA Environmental Protection Agency

FAO Food and Agricultural Organization

FDA Food and Drug Administration

FEPA Federal Environmental Protection Agency

GDP Growth Domestic Product

IDE International Development Enterprise

IDL Instrumental Detection Limit

IPM Integrated Pest management

LLE Liquid- Liquid Extraction

MDL Method Detection Limit

MRL Maximum Residue Limit

MRM Multi residue Method

MSPDE Matrix Solid Phase Dispersion Extraction

ND Non- Detected

PAN Pesticide Action Network

PANUK Pesticide Action Network of United Kingdom

PSE	Pressurized Solid phase Extraction
SBSE	Stir-Bar Sorption Extraction
SNNP	Southern Nations and Nationalities People
SPE	Solid Phase Extraction
SPME	Solid Phase Micro Extraction
UAR	Unidentified Analytical Response
USDA	United States Development Agency
USEPA	United State Environmental Protection Agency

Abstract

Two Organochlorines (endosulfan and DDT), four organophosphorus (dimethoate, malathion, diazinon, chlorpyrifos), and one pyrethroid (deltamethrin), pesticide residues were extracted with ethyl acetate followed by ethyl acetate: n-hexane in the order of (90:10 mL v/v) and (80/20 mL v/v) from tomato and cabbage samples and analyzed by Gas chromatography coupled with flam ionization detector. The analysis was carried out by split injections using capillary column GC-FID in Adami Tulu Pesticide Processing Share Company. The vegetable samples were collected from Dugda Woreda, specific areas called Wolda-Kelina and Shubi-Gemo agricultural sites near Lake Ziway. Linearity for determination of these pesticides from tomato and cabbage samples were evaluated using the external calibration curves of each pesticide at five calibration points (0.25, 0.5, 1.25, 2.5 and 5) µg/mL. However in the analysis, organochlorine pesticides (endosulfan and DDT) and chlorpyrifos from organophosphorus, were not detected by this method in both tomato and cabbage samples in the above calibration ranges. But three organophosphorus pesticides (dimethoate, diazinon, malathion), and one pyrethroid pesticide (deltamethrine) were detected with residual concentrations of 0.35, 0.22, 0.16 and 0.97µg/g respectively in tomato and 0.16, 0.06, 0.19 and1.67µg/g respectively in cabbage samples. The average recoveries for most of the pesticides were in the acceptable range and the detection limit of each detected pesticide is below MRL.

1. INTRODUCTION

1.1. Background of the study

Pesticides are synthetic organic chemicals used for destroying or preventing the activities of harmful insects, weeds, fungus and diseases in the fields, and mites that feed on crops. They are extensively used in agricultural production to check or control pests, diseases, weeds and other plant pathogens in an effort to reduce or eliminate yield losses and preserve high product quality (Bradman and Harley, 2008). According to FAO and UNEPA (1990), pesticides are organic chemicals designed to combat the attacks of various pests and vectors on agricultural crops, domestic animals and human beings. The definitions above imply that pesticides are toxic chemical agents and that are deliberately released in to the environment to combat crop pests and disease. Therefore, in different vegetables and fruits, pesticides are repeatedly applied during the entire period of growth and sometimes even at fruiting stage for better yield and quality (Balso *et al*., 2005).

Farmers around the world use different kinds of pesticides such as organochlorines, organophosphorus, pyrethroids, carbamets insecticides, fungicides and herbicides against the possibility of a devastating crop loss from pests and diseases, as well as to increase agricultural productivity to provide adequate food supply for the increasing world population. Over the past couple of decades, a rapid increase in the quantity and use of pesticides in the agricultural sector has been observed and this growth trend is expected to continue for the next decades due to several socio-economic and technological developments (Kurenrachie *et al*., 2011).

Although pesticides are purposely applied to the environment with aim to suppress plant and animal pests and to protect agricultural and industrial products, the majority of pesticides are not specifically targeting the pest only, and during their application they also affect non-target plants and animals. In addition to this, many pesticides are not easily degradable, they persist in soil, leach to groundwater and surface water and contaminate wide environment. Reports have shown that the estimated annual application of pesticides is more than 4 million tons, but only 1% of this reaches the target pests. The remaining accumulates in the soil, where it may filter in to ground or surface water and prove toxic to micro organisms, aquatic animals, and humans (Gavrilescu, 2005).

According to the report, it is estimated that 20,000 unintentional deaths and 3 million poisonings caused by pesticide miss-use in the third world each year due to lack of training or illiteracy. Therefore, the intensive pesticide application results in several negative effects in the environment that cannot be ignored (Napierska and Podolsk, 2006)

1.2. Pesticide use and poisoning cases in Africa

Although pesticides are important to prevent production loss due to pests in agriculture, they can pose significant environmental and health hazards when they are not properly handled and managed under field conditions. For instance, average pesticide use in Africa is estimated to 1.23 kg per hectare, and this is low compared to 7.17 kg and 3.12 kg for Latin America and Asia respectively. But harmful environmental effects are magnified by the use of banned or unauthorized products, miss handling and higher rate of applications to crops in many countries of the region. Developing countries in general have used fewer pesticides in the past, but pesticide use is expected to grow more rapidly in these countries than in the developed world but the efficiency rates of pesticide applications are even lower than that of fertilizers (Martinez *et al*., 2008).

In addition to this, pesticides that are restricted in developed countries are continue to be applied in developing countries and wrong application techniques, badly maintained or totally unsuitable spraying equipment, inadequate storage practices, the reuse of old pesticide containers for food and water storage have high contributions to the risk of exposure in Africa (Abdela *et al*., 2011). In areas with lax pesticide regulations, farmers may use cheap locally produced pesticides that would be illegal elsewhere (WHO, 2007). A survey of pesticide use among small holder cotton farmers in Zimbabwe found that over half had experienced acute pesticide poisoning symptoms, including skin irritation, eye irritation and stomach poisonings. For instance, persistent organochlorine pesticide endosulfan was introduced in cotton production in West Africa in 2001, as part of a regional program to combat pyrethreoid insecticides resistant in the bollworm Helicoverpa armigera. In the first season of its introduction, cases of acute poisonings including fatalities were observed and official sources in Benin stated that at least 37 people died due to endosulfan poisonings (Sefa-Dedeh, 2009).

1.3. Statement of the problem

The Dugda Woreda is the area where large amount of vegetables and fruits are produced and transported to the central Ethiopia. Farmers in the area traditionally use small scale motorized irrigation system and under these systems they produce vegetables and fruits by using furrow irrigation by lifting up water from the underground or Lake Ziway with motorized pumps (Reta, 2011). In order to minimize diseases and pest pressures, all vegetable growers use crop rotations and pesticides such as organochlorines, organophosphorus and pyrethroids for disease and pest control (Getachew and Mohammed, 2012). As reported by Getachew and Mohammed, (2012), some vegetable growers in the area mix insecticides and fungicides and spray as high as 16 times in a wet season and as high as 8 times during dry season whilst the recommendation is a maximum of 5 times when the worst infestation occurs.

Warm season cultivation after January requires more sprays than cool season and the longer life cycle of vegetables like tomato entails more number of sprays per season. This indicates that there might be high pesticide residues in the soil and on the surface and tissue of leafy and fruit vegetable products such as cabbage and tomato grown in the area (Edessa *et al*., 2013). Since farmers in the area are not aware of the properties of these pesticides, they do not consider the time gap between spraying and harvesting (consumption time) and selling of vegetables after 3-4 days of spraying is a normal practice in most of the areas of the region. They do not also consider the recommended amount of these pesticides per hectare of land, there is also inappropriate disposal of pesticide containers in the farm during and after spraying. As the vegetable farms are found nearby villages, consumers especially children are exposed to harmful chemicals by ingesting directly raw or half-cooked contaminated vegetables like tomatoes. (Deribe *et al*., 2011).

Therefore, this study will be significant to show preliminary data regarding the pollution status of tomato, and cabbage by organochlorine, organophosphorus and pyrethroid pesticides by considering the time gap between spray and harvesting time. It will also contribute for economic development of the country by showing the residue status of these pesticides in export agricultural products. The study will be used as base line information in the aspects of analysis of pesticides residues in food matrix in general and in vegetables in particular.

Therefore, the main focus of this study will be on the assessment of some selected organochlorine, organophosphorus and pyrethroid pesticides in cabbage, and tomato samples which are largely produced in Meki area, specifically in agricultural sites called *Shubi- Gemo and walda- kelina.*

1.4. Objectives of the study

1.4.1. General objectives

The general objective of this study is to assess the residue levels of some selected organochlorines, organophosphorus and pyrethroid pesticides in Dugda woreda fruits and vegetables farms.

1.4.2. Specific objectives

- To quantitatively determine the levels of some selected organochlorines, organophosphorus and pyrethroid pesticide residues in cabbage and tomato growing in the area.
- To compare the residue levels of organochlorines, organophosphorus and pyrethroid pesticides in tomato and cabbage with international maximum residue level (MRL).
- To assess pesticide uses and application procedures of small-scale farmers in the study areas.

2. LITERATURE REVIEW

2.1. Agriculture and pesticide use in Ethiopia

Ethiopia is an agricultural dominant country where 85% of the population is involved directly or indirectly in the agricultural sector. Since agriculture is the dominant sector for the Ethiopian economy, it accounts for about 50% of the GDP and 85% of export revenue and providing livelihood for 85% of the population (World Bank, 2007). The country has a favorable climate, abundant labor, land and water resources and most regions of the country are suitable for the production of a wide range of tropical and subtropical fruits, vegetables and flowers (CSA, 2007-2008). As it is evident from the current development strategies and policies, such as Agricultural Development Lead Industrialization (ADLI) Policy, it is expected that agriculture will continue to dominate the country's economy for long time in the future (EIA, 2012).

The farming system in Ethiopia is dominated by smallholder agriculture which supplies approximately 95% of the production in the country. The smaller agricultural production system is mainly based on traditional inputs. Land preparation is done by oxen-drawn plough or by manually operated tools and seeding is done by broadcasting and weeding which largely depends on intensive family labor. Intercropping and multiple cropping are practiced to cope up with the problems of small land size which currently averaging below 1 hectare per household (Bezabih *et al.,* 2010).

In Ethiopia, although chemical pesticide use was historically low, recent developments resulted from increased food production and expansions in floriculture industry have resulted in higher consumption of chemical pesticides (Asefa, 2011). Current results have showed that about 30-40 percent crop yield has lost to pest and disease annually in the country and in addition to this, the increasing population, shrinking farm size, has resulted in food insecurity. Therefore, the government has considered a viable option to overcome these problems by agricultural intensifications including the increased use of agrochemicals such as fertilizers, pesticides and selected seeds in large amount in vegetable and crop production (Domotrova *et al*., 2006)

2.2. Sources of agricultural pesticides in Ethiopia

At present, most of the agricultural pesticides used in Ethiopia are imported. In most cases pesticides are imported to Ethiopia for agricultural purpose while some amount is imported for health and industrial purposes. Commercial farmers are the major users of pesticides in Ethiopia and they use 80% of the pesticide imported to the country and the remaining 20% of the total imported is used for house hold, health and industrial purposes (Alterra, 2010). Both public and private enterprises are engaged in pesticide importation business and currently about 20 organizations are actively involved in importation and sale of pesticides. Large quantities of pesticides are imported annually to Ethiopia and over 3000 tons of various pesticides that are worth more than 20 million US dollar are imported annually ((Hailemichael and Dalvine, 2009).

In addition to this there is one local pesticide formulation plant in Ethiopia located near Adami Tulu Woreda of Oromia regional state in the Rift Valley, some 170 km south of Addis Ababa. The plant has the capacity to formulate 1500 tons of dust and the same quantity liquid formulation every year by importing active ingredients and solvents from foreign countries. However agricultural intensification and threat of pesticides in its wide spread use together with very little published information on the levels of pesticides in different environmental compartments make individual interventions in food safety ineffective (Malin, 2006).

2.3. Vegetable production and pesticide use by small scale farmers in Rift Valley areas of Ethiopia

The production of vegetables in Ethiopia varies from cultivation of a few vegetables for home consumption to large scale production for the domestic and foreign markets. According to CSA, (2012), the areas covered by vegetables and root crops were estimated to be 359,950.13 hectares with a total production of 24,267581.58 tones in the year 2011/2012. Vegetable production in Ethiopia is scattered throughout the country on patches of land in peasant small holder farms, where as large scale production and processing of vegetables is carried out only by state organizations (Wiersinga and Snels, 2008).

IDE has recently introduced vegetable production in some parts of the Ethiopian highlands but these are on a small-scale mainly to promote increased household consumption due to the need

for irrigation facilities as a pre-requisite for production in this agro-climatic zone. Commercial horticultural crop production is carried out mainly in the central rift valley and eastern part of the country (ACIAR, 2012). The most altering concern is indiscriminate use of pesticides by vegetable farmers in the country due to their easy availability, relatively cheap cost and ease of application. Thus the increase use of pesticides in agriculture has resulted in the occurrence of residues in food commodities that has always been a matter of serious concern especially when these commodities are consumed fresh. Actually the pesticide residues in food and crops are direct results of application of pesticides to agricultural fields and to a lesser extent from pesticides contaminating the soil (Chowdhury *et al*., 2012).

This indiscriminate and overuse of harmful pesticides can contaminate the environment and ultimately entered into the food chain which cause severe damage to humans health, fishes and many other animals, conducting to death (Barr *et al*.,2006). Current reports have shown that, in addition to Organophosphates (i.e. chlorpyrifos, diazinon, etc.), pyrethroids (i.e. cypermethrine, Deltamethrine etc.) and carbamates (i.e. carbofuran, aldicarb, carbaryl, etc.), globally banned persistent organochlorine pesticides such as DDT and endosulfan are being used across the country and widely applied in vegetables including tomato and cabbage particularly in rift valley areas of the country (Abdurrahman *et al*., 2013). According to all African.com report (2015), the Ethiopian agricultural experts have discussed on the negative impacts of pesticides in a recently held four day workshop, and one of the expert, Mengistu Wondafrash said that globally banned persistent pesticides such as DDT and endosulfan are being used for agricultural purpose across the country due to lack of awareness and information in the society (Daniel, 2015).

Most studies in the Ethiopian Rift Valley regions indicate that the awareness of farmers on the proper handling, storage and application of pesticides is very low. Farmers were seen spraying pesticides with bare feet, without other protective devices, near water bodies sometimes even near public water supply points. There are also some reports showing that the illegal use of pesticides which are banned from agricultural sector like DDT. Although DDT is banned for use in agricultural purpose, recent survey conducted in the area shows that DDT is used as insecticide by farmers (PAN-UK, 2008-2012).

Some of the reasons for improper use of pesticides by growers include lack of knowledge on pest identification and unable to use the correct pesticide at the right dosage, and others.

Non-pesticidal control options such as biological, cultural, and varietal control developed in research centers have not reached farmers because of poor research-extension systems (Habtu and Tesfaye, 1985; Gashawbeza and Tsedeke, 1994). Generally the impacts of pesticides in Ethiopia are much more aggravated by the limited knowledge among users on safe practice, toxicological and chemical properties of these substances.

Even worse, less is known about the long term and indirect effects of pesticides on rural and urban communities as well as on local and national food production systems. In Ethiopia, regulating food safety is a share responsibility of the Ministry of Health, Ministry of Agriculture and Quality and Standard Authority of Ethiopia. But the studies of the levels of pesticide residues in food are limited in the country and the impacts of pesticides in a given environment have not been clearly identified, assessed, and complied and also there is no system for risk monitoring and communication (Thomas *et al*., 2006).

Therefore, the protection of consumer health and the environment from the Pesticides is of primary importance in Ethiopia. Thus, this study which is aimed at generating information on residue levels of vegetables collected from Meki agricultural areas will provide baseline data for possible control and effective use of pesticides in accordance with the country's National Implementation Plan for the Stockholm Convention and FAO's Environmental Management Toolkit.

Table1: Environmentally significant properties of the Pesticides frequently applied by smallholder farmers in the study area.

Active ingredient	**Types**	**WHO Class**	**Molecular formula**	**Molar mass (gm/mol)**	**Half life in the Soil (days)**	**Water Solubility at 20°c in mg/L**	**Vapor pressure in mmHg at 20°c**
Chlorpyrifos	Organophosphorus	II	$C_9H_{11}Cl_3NO_3PS$	350.5	35-56 days	1.4	$2x10^{-5}$
Diazinon	Organophosphorus	II	$C_{12}H_{21}N_2O_3PS$	304	11-21 days	60	$9x10^{-5}$
Diamethoate	Organophosphorus	II	$C_5H_{12}NO_3PS_2$	229	2-5 days	23,800	$1.8x10^{-6}$
Malathion	Organophosphorus	III	$C_{10}H_{19}O_6PS_2$	330	3-6 days	145	$8.x10^{-6}$
DDT	Organochlorine	II	$C_{14}H_9Cl_5$	354.5	2000 days	0.0055	$1.8x10^{-7}$
Endosulphan	Organochlorine	II	$C_9H_6Cl_6O_3S$	407	30-70 days	0.32	$6.2x10^{-6}$
Deltamethrin	Pyrethroid	II	$C_{12}H_{19}Br_2NO_3$	384.8	23 days	0.0002	$9x10^{-11}$

I= extremely hazardous, II= highly hazardous, III = moderately hazardous

Sources: Field survey and Environmental and Ecological chemistry vol.III, Tomlin, C. (Ed.) in the Pesticide Manual, British Crop Protection Council, 2000 and WHO, 2009.

2.4. Ways of Pest Control Mechanisms

Commonly there are three kinds of approaches that are used to eliminate or decrease the amount of pests of crops and vegetables: **i** The first approach is called ecological approach which gives long lasting protection of pests by developing control agents based on the knowledge of the pest life cycle and ecological relationships. Such ecological agents may be other organisms or chemicals that work in different ways and either they are highly specific to the pest species being fought or they manipulate one or more aspects of the ecosystem. This type of control system emphasizes the protection of people and domestic plants and animals from damage of pests, instead of totally eradicating of the pest organisms. Therefore, this approach used as the pest control mechanism while maintaining the integrity of the ecosystem (Abate, 2006).

ii. The second approach is the Integrated Pest Management system (IPM) which is a pest management system used based on the environment and the nature of the pest species in question. It utilizes all suitable techniques and methods of pest control in compatible to maintain

the pest at levels below causing economic loss. Thus pests are treated as part of the whole ecosystem rather than as isolated occurrences (Lowell, 2008). In the IPM system, chemicals may be applied only as last resort as the time when pests are most vulnerable, rather than routinely by calendar schedule. IPM is an ecologically sound, environmentally friendly and economically affordable pest management approach that employs optimum blends of control measures to keep pest members below economic level (Tsedeke *et al*., 2006).

IPM focuses on long term prevention of pests and their damage. It does not exclude the use of pesticides, but predicates that all other means must be explored and pesticide use should be considered as a last resort. IPM is more effective than synthetic pesticides and generally requires less capital investment and can be used preventatively to eliminate or minimize the need for applying pesticides. For example, African bollworm in beans and vegetables like tomato and pepper, and fruit worms in tomatoes grown under irrigation in Ethiopia can be protected by this approach (Nigatu, 2010). **iii** The third approach is by using a chemical treatment. Although this approach has much success in controlling pests, it gives only a short- term protection. Furthermore; the chemicals often have side effects by damaging other non-target organisms (Asferachew and Tadesse, 2008).

2.5. Classification of pesticides

Pesticides can be classified differently. One way in which pesticides can be classified is by the type of pest or diseases against which they are very effective like insects, fungi and weeds. Function wise they are divided in to herbicides (protection against weeds), insecticides (against insects), fungicides (against fungi), and others. Herbicides**:** used to kill weeds and other plants growing in places where they are unwanted. Insecticides: Employed to kill in sects and other arthropods, and fungicides to kill fungi. Insecticides are mainly classified as Organophospohrus pesticides (Ops), Organochlorines (OCs), Pyrethroids and Carbamates (WHO, 2000-2002).

2.5.1. Organochlorine pesticides (OCPs)

Organochlorine pesticides are chlorinated organic compounds used predominantly for agricultural purpose to kill insects. Being chemically stable and highly persistent in the environment, they have been banned in most countries of the world.

These organochlorine pesticides classified as DDT and its metabolite, Cyclodienes like endosulfan and endosulfan sulphate and Hexachlorocyclohexanes (Donaldson *et al*. 2010). Persistent organochlorine pollutants such as: aldrin, chlordanes, dieldrin, endrin, heptachor, heptalor epoxide, hexachlorobenzene (HCB), mirex, dichlorodiphenyltrichloroethane (DDTs), toxaphene, and hexachlorcyclohexanes (HCHs) are some of the most common organochlorine pesticides. They have similar structures, showing chlorine-substituted aliphatic or aromatic cyclic rings and because of the structural similarities, these pesticides share certain physicochemical characteristics such as persistence, toxicity, bioaccumulation, and long-range transport potential (Sherwood *et al*., 2005).

As a result, the Stockholm Convention considered some organochlorine pesticides as environmental hazards and listed them as persistent organic pollutants (FAO/WHO, 2004). These chemicals are difficult to degrade into less hazardous substances in the environment. They are lipophilic compounds that tend to bioaccumulate in fatty tissues through the food chain. These pesticides are water insoluble and semi volatile, enabling their entry in the atmosphere and transport over long distances globally, mainly by air mass movements. They can reach polar of high mountainous regions and are effectively deposited in cold regions by snow through the phenomenon of cold condensation and global distillation (Wanja and Mackay, 1995).

Dichlorodiphenyltrichloroethane (DDT) is the common name of 1, 1, 1-trichloro-2,2-di-(4-chlorophenyl) ethane. Technical-grade DDT is a mixture of up to 14 compounds. The active ingredient is *p,p*-DDT (65-80%). The other compounds includes 15-21% of *o,p'*-DDT, up to 4% of *p,p*'-DDD and 1-(p-chlorophenyl) -2,2,2-trichloroethanol, and traces of *o,o'*-DDT and bis(p-chlorophenyl)sulfone. DDT easily degrades in to dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), which are more persistent than the parent compound (Hengsdijk and Jansen, 2006).

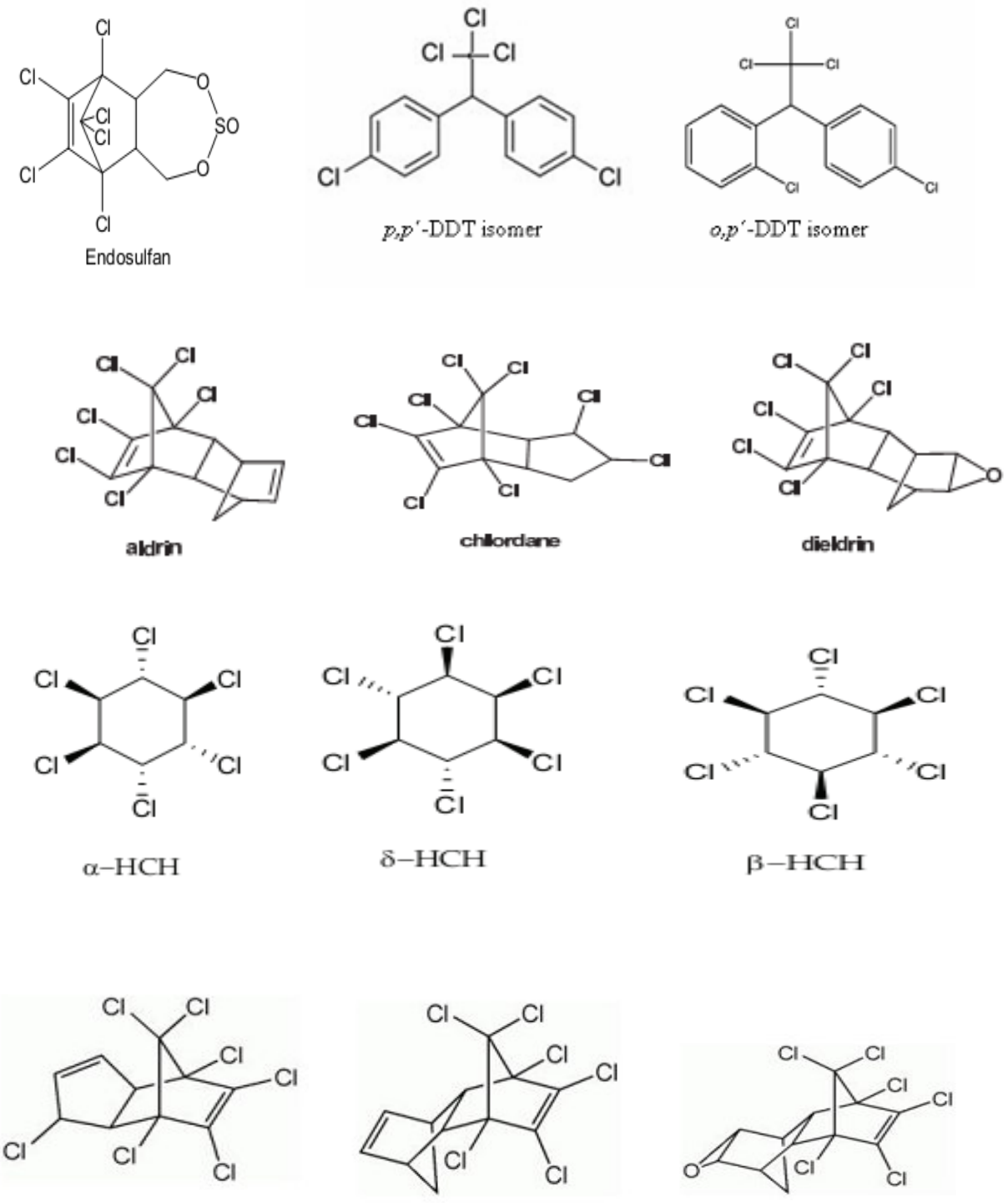


Figure 1: Structures of some **organochlorine** insecticides (Muri and Howard, 2006).

2.5.2. Organophosphorus pesticides and their mechanisms of toxicity

Organophosphorus pesticides or organophosphates (Ops) are from a large group of chemicals used over the past 60 years for protecting crops, livestock, and human health and as warfare agents. Organophosphorus pesticides such as dichlorvos, methyl parathion, chlorpyrifos, diazinon, demeton-5-methyl, fonfos, chlorfenvinphos, fenitrothion and Malathion are the principal group of insecticides used to protect vegetable from pests in industrial plantations and vegetable cultivation. Among those, pesticides such as diazinon, chlorpyrifos and methidathion are complex molecules and generally have longer lasting residues than many of the aliphatic or phenyl organophosphorus pesticides (Bolognesi, 2003; Maroni et al., 2000).

Organophosphorus pesticides are among the most acutely toxic pesticides mainly used as insecticides with most of these chemicals classified by the US Environmental protection agency as toxicity class I-highly toxic or toxicity class II-moderately toxic. The toxicity of Ops depends on their chemical structure, metabolism in target organism, concentration, mode of application, degree of decomposition and mode of entering into the organisms. Unlike other man- made chemicals, Op pesticides can affect a large proportion of the human population as a result of exposure through domestic use, proximity to agricultural activities and consumption of contaminated food and water (EPA, 2006).

The best described op toxic effects are the neurological symptoms following acute poisoning of an acetyl cholinesterase enzyme which is the primary target of these chemicals. AChe is found in synaptic membranes, where it degrades, through its hydrolytic activity the neurotransmitter acetylcholine producing choline and acetate, a reaction important for the regulation of synaptic activity in the central and peripheral neural system (Koprucu *et al*., 2006). Organophosphorus pesticides inhibit AChe by forming covalent bond between Op and the active site of AChe. Spontaneous hydrolysis of organophosphorus pesticides from the active site is very slow sometimes irreversible resulting in long-term toxic effects. The primary Ops are altered in such a way that spontaneous hydrolysis of the OP-AChe complex is accelerated (Gupta, 2006).

The most informative data regarding potential human carcinogenicity come from epidemiological studies of the exposed population. Chronic occupational exposure to Ops has been linked to increased risk for cancer development such as non- Hodgkin lymphoma and some

types of leukemia. Possible health problems associated with chronic pesticide toxicity include cancers, congenital malformation, neurological disorders, infertility, blood dispraises, impotence, immunological disorders, liver and kidney damage, skin alteration and worsening of existing health conditions (Eskenazi *et al.,* 2008).

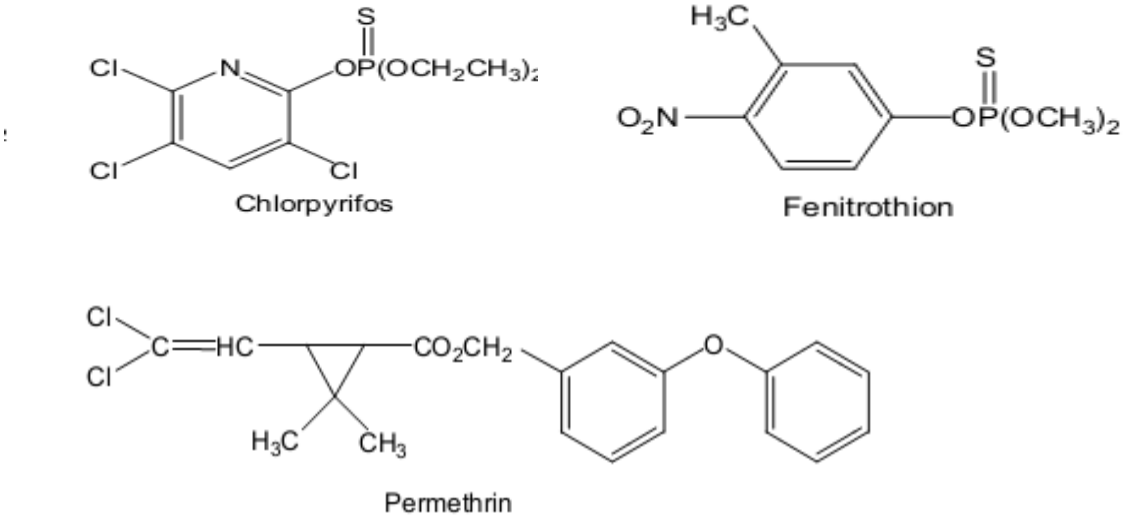


Figure 2: Structures of some **organophosphorus and pyrethroid pesticides**

Pyrethroid Insecticides: Pyrethroids insecticides are considered as contact poison insecticides that affect the insect nervous system and depolarizing the neuronal membranes. Insecticides such as cypermethrin, deltamethrin, perimethrin and acrinethrin, etc are pyrethroid insecticides. Deltamethrin is immobile in the environment because of its strong adsorption on particles and its insolubility in water however it has risks to the ecosystem in which it is applied even at very low rates of application. Cypermethrin is highly toxic to fishes, bees and aquatic insects but in human it is deactivated by enzymatic hydrolysis to several carboxylic acid metabolites, which are eliminated in the urine (Sereda and Meinhardt, 2006).

2.6. Environmental fate and transport of pesticides

The biological activities of a pesticide to the target species is greatly influenced by its physical and chemical properties. The physical properties of pesticides in particular determine the pesticide mode of action, dosage, mode of application and the subsequent environmental effect and these physical properties of the pesticides vary greatly according to their chemical nature and

formulation. The major physicochemical properties that determine the fate and transport of pesticides in the environment are: water solubility, volatility of the pesticide in air, adsorption in soil and sediments, bioaccumulation and persistency (Baselt, 2008).

The application site (crop or soil) and method, the type of use (agricultural or non- agricultural), the formulation (granules or suspended, powder or liquid) and the application amount and frequency can also influence the transport of pesticides in the environment (Wenli, 2012). Sometimes, releasing pesticides into the environment can be harmful, because not the entire applied chemical reaches the target site. For example, runoff can move herbicides away from target weeds. The chemical is wasted, weed control is reduced, and there is more chance of damaging other plants and polluting soil and water or some of the pesticide may drift downwind and outside of the intended application site. Processes such as adsorption, transfer, breakdown and degradation may affect what happens to pesticides in the environment and each of these processes is explained in the following sections (Zhang *et al*., 2009).

Solubility of pesticides: The significance of solubility for environmental fate pesticides is that, a pesticide which is very soluble in water will tend not to accumulate in the soil or plant because of strong polar nature. **Octanol/water partitioning coefficient-Kw (P^{Kw}):** Partitioning coefficient is a measure of the ratio of the dissolved mass of the substance between equal layers of n-octanol and water.

$$kow = \frac{concentration\ in\ n-octanol\ phase}{concentration\ in\ water\ phase}$$

Values of Kow for organic pesticides are large and mostly expressed as P^{Kow}. Kow is a good indicator of bioaccumulation of pesticides in organisms and food chains. Pesticides with positive correlation with P^{Kow} are more likely to have bioaccumulation effects to organisms and food chains and pesticides with low P^{Kow} values greatly indicate the likely systemic translocation of such pesticides or their metabolites in the plant. Therefore Polar pesticides tend to be more soluble in water and hence low values of P^{Kow}. For the general physical factors P^{Kow} will increase when physical properties such as molecular surface area, molar volume, molecular weight and density of pesticides increase (Zacharia, 2011).

Soil adsorption coefficient-Koc/Kd: Adsorption of pesticides on soils and sediments is a major factor that determines the destination of pesticides in the environment and their eventual degradation processes. Most pesticides are non- polar and hydrophobic. These non- polar pesticides tend to be pushed out of water onto soils and sediments which contains non- polar organic matters. Kd is called the sorption coefficient and it measures the amount of pesticides adsorbed onto the soil per amount of water without considering the organic matter content of the soil. The values for Kd vary greatly because the organic matter content of the soil is not considered in the equation (Abhilash, 2009).

The preferred parameter to determine the soil ability to absorb pesticides is Koc since it considers the organic matters content of the soil. Koc is the ratio of the mass of a substance adsorbed onto a unit mass of soils, relative to the mass of the substance remaining in water solution. Koc is also a unit less parameter and its value is dependent on the organic matter content of the soil, polarity of the pesticide and soil P^H.

$$\text{kd} = \frac{\text{concentration of a chemical in the soil}}{\text{concentration of a chemical in water}}$$

$$koc = \frac{\text{kdx100}}{\%\text{organic carbon}}$$

Vapor pressure (VP): The vapor pressure of a substance is the measure of how easy it can be volatile and turn into vapor (gas state). For pesticides the ease with which it can be volatile may be considered advantageous with respect to a particular mode of action on one hand but it can be of negative influence on the other hand. For example, a pesticide with a fumigant mode of action can have a useful penetrating power and thus it is advantageous to have high vapor pressure. However, a high vapor pressure can cause a vapor drift and environmental pollution. Pesticides with high vapor pressure need to be handled in such a way that the vapor pressure do not escape into the atmosphere. A pesticide with low vapor pressure does not move into the air, so there is a potential to accumulate if it is water soluble, but if it is not water soluble, the pesticide may accumulate in the soil and plant (Sannino, 2008).

Absorption is the uptake of pesticides and other chemicals into plants or microorganisms. Most pesticides break down once they are absorbed. Pesticide residues may be broken down or remain

inside the plant or animal and be released back into the environment when the animal dies or as the plant decays. Some pesticides stay in the soil long enough to be absorbed by plants grown in a field years later. **Degradation or Breakdown Processes:** Degradation is the process of pesticide breakdown after application. Pesticides are broken down by microbes, chemical reactions, and light or photo degradation. This process may take anywhere from hours or days to years, depending on environmental conditions and the chemical characteristics of the pesticide. Pesticides that break down quickly generally do not persist in the environment or on the crop. However pesticides that break down too rapidly may only provide short-term control (Antonou *et al*., 2008).

2.7. Methods of extraction and analysis of pesticides in food samples

Most of the analysis is done by taking part of the object under study and analyzing it in the laboratory or at the site. The first step is sampling, where the sample is obtained from the object to be analyzed. The collected samples should represent the original object (Wiley, 2003).There are different sampling methods and their choice depends on the nature of the bulk material, the system investigated, and the purpose of analysis. **Random sampling** is used when the bulk material has an equal chance of being selected. **Systemic sampling** is achieved by taking a portion of the bulk material on a regular basis in space or time. It is used when the bulk material is distributed in a zone of priority, either in reality or schematically (Winefordner, 2003).

Stratified Sampling is used when a system contains several distinctly different areas, these may be sampled separately, in a stratified sampling scheme. The target population is divided into different regions or strata. The strata are selected so that they do not overlap each other and then random sampling is done within each stratum. The strata in a stratified scheme do not necessarily have to be obviously different. The area may be divided into arbitrary subareas. Then a set of these are selected randomly. Each of these units is then sampled randomly. **Grab sample***:* A grab sample is a discrete sample which is collected at a specific location at a certain point in time. If the environmental medium varies spatially or temporally, then a single grab sample is not representative and more samples need to be collected (Tratter, 1990).

Composite sample*:* A composite sample is prepared by thoroughly mixing several grab samples. A composite sample may be made up of samples taken at different locations, or at different points in time and it represents an average of several measurements and no information about the variability among the original samples is obtained. When the sampling medium is very heterogeneous, a composite sample is more representative than a single grab sample and it may be used to reduce the analytical cost by reducing the number of samples. A composite of several separate samples may be analyzed and if the pollutant of interest is detected, then the individual samples may be analyzed individually (Gilbert, 1987). The composited farm sample reaches, the laboratory may have to be reduced in size and a reduced sample is prepared by taking a representative portion of the original sample, usually by a mixing and dividing process.

After a reduced sample or a subsample is subjected to the laboratory processes needed to prepare it for analysis (grinding, dividing, mixing) it is referred to as a test sample. From this test sample, test portions with proper size and concentration are removed for the analysis and this test portion is readily run on the instrument or to be analyzed by the chosen method. Often this test portion is dissolved, digested or extracted to obtain a test solution and sometimes the test solution is subdivided into equal portions, often to allow replication of the analytical method.

Sample Preservation: Commonly encountered problems are biodegradation, oxidation-reduction and volatilization. Storing the sample at low temperature is always recommended to reduce volatilization, chemical reaction, and biodegradation.A preservation temperature of $4^{o}C$ is most commonly used, because ice storage is inconvenient and a lower temperature than this may freeze the water and separates the organic phase from the aqueous.

2.8. Isolation (extraction) of pesticides from vegetable matrix

Since most samples are not ready for direct introduction in to instruments, the next step is sample preparation. In determination of trace contaminants in complex matrices such as food, often requires extensive sample extraction and preparation before instrumental analysis. Even though there is a considerable progress in the development of methods for preparing samples for analysis and for final determination of analytes, the analysis of pesticides in biological samples is a challenge to analysts due to the complexity and the diversity of matrices present in the material

and the low concentration of pesticides in the samples of fruit and vegetables. Therefore, before the final determination of these pesticides, the target analytes must be isolated from their matrices. It is very important that several stages of the analytical procedure and the procedure as a whole are validated in order to ensure compliance with the requirements defining the procedure and to assess its usefulness.

The sample preparation stage and the operations involved in it can affect the final result (FAO, 2006). So, whether the analysis provides the desired information about the sample therefore, depends on its proper and correct preparation. It is extremely important that the sample of the material for the analysis is homogeneous and representative. Generally, the sample preparation techniques consist of a number of steps and in the case of fruits and vegetables, steps like, removal of surface contaminants, drying, breaking up/crushing/ are important (Winefordner, 2003).

The aim of any extraction process is the isolation of analytes of interest from the selected samples by using appropriate extracting phase. Pesticides from environmental samples are preferably extracted using solid phase extraction (SPE), solid phase micro extraction (SPME), liquid-liquid extraction (LLE), matrix solid phase dispersion extraction (MSPDE). In all cases, once a liquid extract has been obtained it is subsequently subjected to purification or clean up step which is usually performed by SPE or LLE (Chai, 2013).

2.8.1. Solid –Liquid Extraction

Solid-liquid extraction is the most widely used procedure in the analysis of pesticides in the solid sample. It is based on the contact of a certain amount of sample with appropriate solvent. The solvent must penetrate inside the pores of the solid sample particulates to achieve desorption of the analytes bound to the matrix active sites. Then the analyte have to diffuse through the matrix to be dissolved in the extracting solvent. The analytes must diffuse through the solvent to leave the sample pores and finally should be swept by the external solvent.

The proper selection of the solvent to be used is a key factor in solid-liquid extraction procedures in addition to other factors such as temperature and pressure that have an important influence on the extraction efficiency. Working at high temperature and pressure facilitates the increase in solubility of the analytes in the solvent and helps the solvent to penetrate the sample pores.

Depending on the strength of the interaction between the analyte and the sample matrix, the extraction will be performed in higher or lower temperature and pressure.

2.8.2. Pressurized Solid Phase Extraction (PSE)

Supper critical fluids can be considered as a hybrid between liquid and gas and possesses ideal properties for the extraction of pesticides from solid samples. Pressurized solid phase extraction (PSE) uses solvents at higher temperatures and pressures to accelerate the extraction process, and the higher temperature increases the extraction kinetics, whereas the elevated pressure keeps the solvent in liquid phase above its boiling point, leading to a rapid and safe extractions. This technique is easily applicable for extraction of pesticide from any kinds of solid samples and the higher pressure used allows to perform a very efficient extraction in short time. Supper critical fluid extraction has been widely used for the extraction of pesticides from solid samples due to the effectiveness and selectivity of the extraction and to the possibility of direct coupling to the chromatographic techniques (Kin, 2009).

2.8.3. Liquid-Liquid Extraction (LLE)

Analytes in solutions or liquid samples can be extracted by direct partitioning with an immiscible solvent. Liquid-liquid extraction is based on the relative solubility of an analyte in the two immiscible solvents and is governed by the equilibrium distribution/ partitioning coefficient. Extraction of the analyte is achieved by differences in the solubilizing power (polarity) of the two immiscible liquid phases. Liquid-liquid extraction has been widely used for the extraction of pesticides from aqueous liquid samples (Legesse, 2012).The efficiency of the process depends on the affinity of the analytes from the solvents, on the ratio of volumes of each phase, and on the number of successive extractions.

Hexane or cyclohexane is typical organic solvents used for extraction of non-polar pesticides such as organichlorine and organophosphorus, and dichloromethane or chloroform for medium polarity pesticides such as triazines or phenyl urea. In order to increase the efficiency of extraction by this method, it is better to use mixtures of solvents or by adding salts (salting-out effect). LLE is one of the most common methods of extracting, particularly for organic

compounds from aqueous matter. In this study liquid-liquid extraction techniques were used by using ethyl acetate and n-hexane as extracting and clean up solvents (Filho *et al*., 2011)

2.9. Extract Purification or Cleanup

The isolation of anaytes from biological samples involves a certain cleanup stages which ensure a certain degree of isolation. Cleanup refers to a step or series of steps in the analytical procedure in which the bulk of the potentially interfering co-extractives are removed by physical method. During extraction, the solvent comes in contact with the matrix to enable extraction of the pesticide along with some of the constituents of the substrate matrix also get solublized. The removal of these interfering co-extractives from extracts is called cleanup. Extract clean up is essential and should always precede analysis of the extract. The usual techniques for cleaning up fruits and vegetable extracts are: Solid phase extraction (SPE), Solid phase micro extraction SPME), Matrix solid phase dispersion extraction (MSPDE), Stir-bar sorption-extraction (SBSE), Adsorption chromatography and Gel-permeation chromatography (Mawussi *et al*., 2009).

Solid Phase Extraction: Solid phase extraction is based on the difference affinity of target analytes for two different phases. In SPE: a liquid phase, liquid sample or liquid sample extract is loaded on to a solid sorbent. Based on the polarity of the sorbent, those compounds with higher affinity for the sorbent will be retained on it, and the others will passes through it. If the target analytes are retained, they can be eluted using a suitable solvent with a certain degree of selectivity. SPE can be used directly as an extraction technique for liquid matrices or as a cleanup technique for solvent extracts and it always consists of three to four successive steps (Abhilash, 2007).

Conditioning: The solid sorbent should be conditioned using an appropriate solvent. This step is crucial as it enables the wetting of the packing materials and the solvation of the functional groups. In addition it removes possible impurities initially contained in the sorbent or in the packing. **Loading**: The liquid sample extract are loaded to the sorbent. Usually target analytes are retained together with other components of the sample matrix. The sample may be applied to the sorbent by gravity, pumping, aspiration by vacuum or by an automated system. The sample flow rate through the sorbent should be low enough to enable efficient retention of the analytes and high enough to avoid excessive retention.

During this step the analytes are concentrated in the sorbent. Even though the matrix components may also be retained by the solid sorbent, some of them could pass through enabling some matrix separation of the sample (Juan-Garcia *et al.*, 2004).

Washing: The third step is washing of the solid sorbent with an appropriate solvent having low elution strength to eliminate matrix components which have been retained by the solid sorbent without displacing the analytes. **Elution**: The final step is the elution of the analyte of interest by an appropriate solvent or mixture of solvents without removing the retained matrix components. The solvents volume and the flow rates should be correctly adjusted to ensure efficient elution (Hennion, 1999). Understanding the mechanisms of interaction between the sorbent and the analyte is a key factor on the development of the SPE method. The purification of organic sample extracts is performed by SPE on to polar sorbents and the mostly used sorbents are polar sorbents (silica, alumina, florisil etc.); non-polar sorbents (octyl-silica and octadecyl-silica); ion exchange sorbents and affinity sorbents (Fontanals *et al*., 2005).

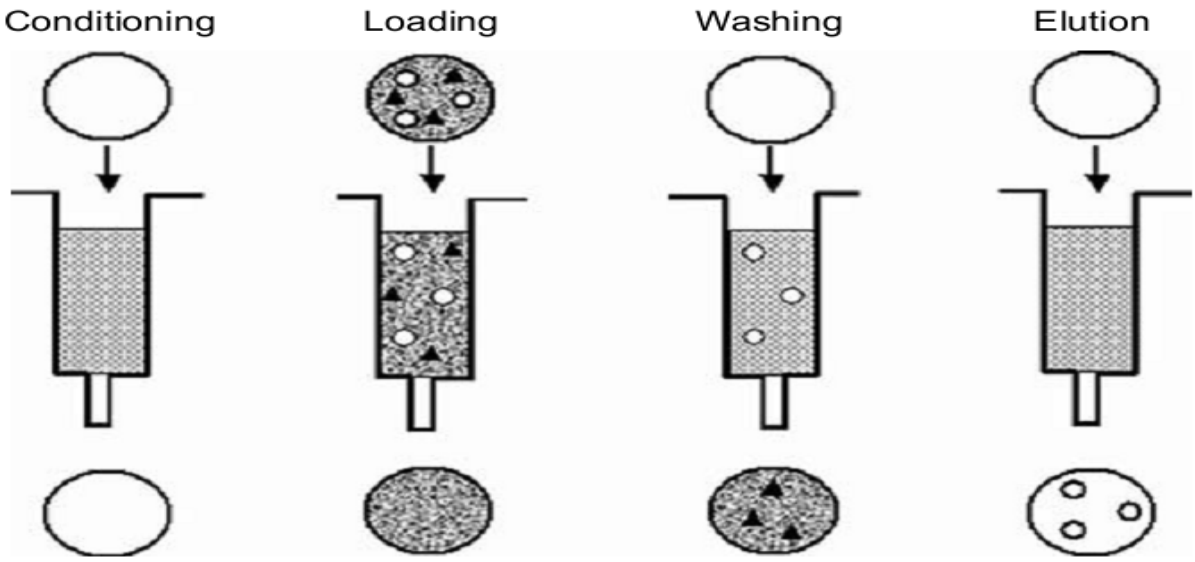


Figure 3: Solid phase extraction and clean up steps

Source: Taylor and Francis (2008). International Standard book on the: Analysis of Pesticides in Food and Environmental Samples. http://www.taylorandfrancis.com.

2.10. Identification and determination of analytes

The last stage in the analytical procedure is identification of compounds and their quantitative determination using appropriate instrumentation. The choice of final determination technique depends above all on the properties of the analytes. Pesticides cannot be treated as a homogeneous group of specific environmental contaminants, because they differ in their physicochemical properties. The usual techniques for final determinations of pesticides include capillary gas chromatography (GC) and high performance liquid chromatography (Ferrer *et al*., 2005). Pesticides such as organophosphorus (OPs), organochlorines (OCs) and pyrethroides are commonly analyzed by using simple gas chromatographic techniques equipped with a suitable column and detectors. Different analytical techniques have been used or the determination of pesticide residues in various vegetables (EU, 2006).

Most of these instruments use gas chromatography coupled with different detectors such as flame ionization detector (FID), electron capture detector (ECD), mass spectroscopy (MS) and so on. Generally the following techniques are employed to determine pesticides in fruits and vegetables: MS (mass spectroscopy) for determination of pesticides of various classes. ECD (Electron Capture detector) is highly sensitive in relation to compounds containing electronegative atoms and generally used for quantification of organochlorine pesticides. FID (flame ionization detector) used for the determination of organophosphorus, volatile organochlorines and pyrethroid pesticides (Tariq *et al*., 2007).

2.11. Multi residue method (MRMs)

These methods are designed to identify and quantify a number of pesticides and their toxicologically significant metabolites simultaneously in a range of foods. Multi residue methods record the presence of unidentified chemicals known as unidentified analytical response (UAR). MRMs contain the steps of preparations, extractions, clean up, chromatographic separations and detections (Nguyen *et al*., 2007). All MRMs used today in the United States are based on either GC or HPLC as determinative steps of the 10 MRMs routinely used by FDA and USDA and 8 relay on GC as the determinative steps such as Luke method for non fatty food types for OC, OP, and ON, Mills methods for fatty food types for OC, and OP (Major, 2007).

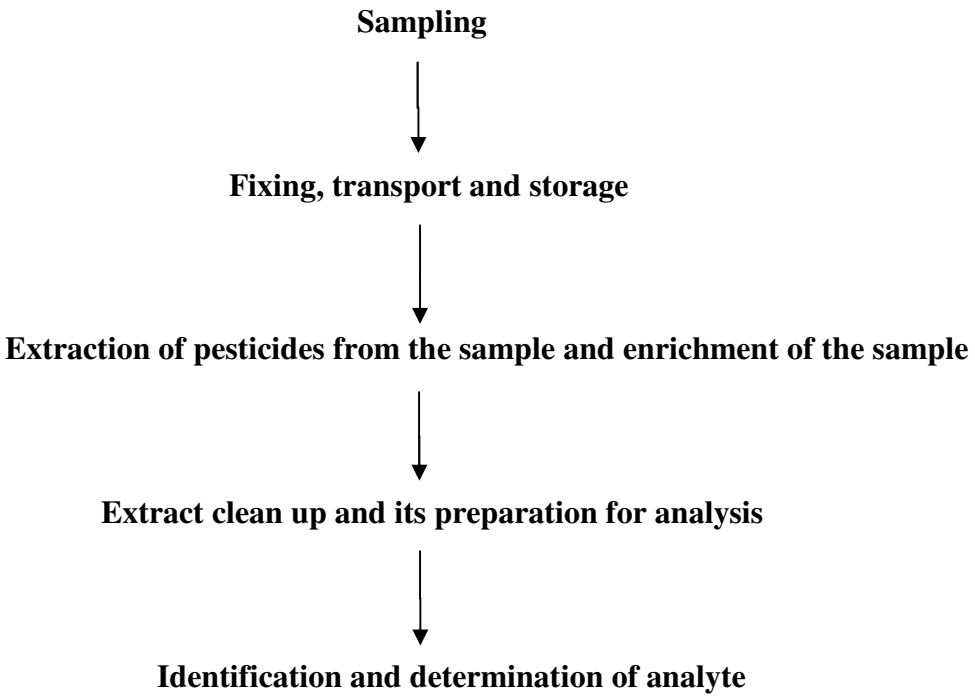


Figure 4: The main stages in analytical procedures for the determination of pesticides in samples of fruits and vegetables. Source: US- pesticide analysis manual.

2.12. Method Validation

Validation is the process where one or more individual chemists test the suitability of a particular method for collecting analytical data. ISO (2005) defines validation as the confirmation via the provision of objective evidence that the requirements for specifically intended use or application have been met, so method validation is the process of defining an analytical requirement and confirming that the method under consideration has performance capabilities consistent with what the application requires (Huber, 2010).

Therefore method validation is an essential component of the measurements that a laboratory makes to allow it to produce a reliable analytical data. The effort expended in validating analytical methods serves to validate the results of some sample analyses. Validation is performed to verify that the method is fit for the purpose, which means that the desired commodities and analytes are evaluated to achieve acceptable recoveries, reproducibility and detection limits (EU, 2009). The method validation study for quantitative analytical methods is important to determine parameters such as limit of detection and limit of quantification,

precision, accuracy and percentage recoveries of the method. In this study the accuracy (percentage recovery), precision (repeatability and reproducibility), limit of detection and limit of quantification were determined for both tomato and cabbage samples (Aysal *et al*., 2007).

2.12.1. Accuracy and precision

Accuracy is the closeness of a measured value (average measured value) to the true accepted value. The degree of accuracy is an indication of the presence or absence of systematic error or bias in the method used. This systematic error may occur during sampling or during extraction (separation) procedures or both. The recovery of the method can be determined either by using internal standard or standard addition method. The accuracy was determined by calculating the percentage recovery of each pesticide residues in each vegetable samples and sample spike.

$$\%R = \frac{C\ spike - C\ sample}{Spike\ conc.} x100$$ (Kumari 2008).

C spike= concentration in the spiked sample

C_{sample} = concentration in the sample

2.12.2. Precision

The precision of a method is the measure of agreement or closeness of analyte concentrations to each other when the analyses are performed using identical conditions. It is a measure of random errors and may be expressed as repeatability and reproducibility. Repeatability is the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment with in short intervals of time. Reproducibility is the closeness of agreement between test results obtained with the same method on identical test material in different laboratories with different operators using different equipments (IAEA, 2009). For a simple assessment of repeatability a minimum of five replicate sample determinations should be made together with a simple statistical assessment of results including the percent relative standard deviation). It is expressed and determined mathematically as the standard deviation of the results of spiked samples in replicate analysis (Getenga *et al*., 2004)**.**

$$SD = \sqrt{\frac{[\sum(X - X1)2 + \cdots + (X - Xn)2]}{n - 1}} \qquad RSD = \frac{SD}{X}100$$

2.12.3. Limit of detection and limit of quantification

Limit of detection is defined as the amount expressed in units of concentration that describes the lowest concentration level of the substance that an analyst can determine to be statistically different from an analytical blank (Garcia *et al*., 2007). Limit of detection may be divided into two components, instrumental detection limit (IDL) and method detection limit (MDL). Each instrument has a limitation on the amount of an analyte that it can detect and this limitation can be expressed as instrumental detection limit (IDL). It is defined as the smallest amount of an analyte that can be reliably detected or differentiated from the background on an instrument (EPA, 2006).

IDL depends on several factors such as sensitivity of the detector for the analyte of interest and instrumental noise of various origins. As the sensitivity increases, the IDL decreases and as the instrumental noise decreases the IDL also decreases. Method detection limit (MDL) is the term that should be applied to extract an analysis methods developed for the analysis of specific analytes within the matrix and it is defined as the smallest amount of an analyte that can be reliably detected or differentiated from the background for a particular matrix by specific method (Corley, 2012).

Limit of quantification (LOQ) is the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated conditions of the experiment. There are different ways of determinations of LOD and LOQ such as **blank determination**, **signal to noise ratio** and **linear regression methods.** Method Detection Limit (MDL) is mostly calculated by blank determination method as three times the standard deviation of a blank spiked with the mixture of pesticide standards at a low level concentration with three or more replicate injections and all the extractions and clean up procedures should be applied to these spiked blank samples (Wood, 2006). The maximum detection limits values obtained should be below the first calibration level for each standard on the calibration curve.

The limits of quantification (LOQ) can be calculated by blank determination method from the limit of detection as ten times the standard deviation of the responses of the fortified blank which is equal to three times of the values of method detection limit (FDA, 2011).

$MDL = 3S_B$ MDL=method detection limit

$LOQ = 10S_B = 3\text{MDL}$ LOQ= limit of quantification

S_B= the standard deviation of the response

In this study the method detection limit and quantification limit were calculated by blank determination method by spiking a blank at 0.25µg/mL with previously prepared pesticide mixture and then extraction, clean up and replicate injection into the GC-FID. The standard deviation and precision of the results of these injections for each pesticide were determined.

3. MATERIALS AND METHODS

3.1. Description of the study area

The study was conducted in the central rift valley areas of Ethiopia in Dugda Woreda, East Showa Zone of Oromia regional state. Dugda Woreda is located approximately between 7^0 58' latitude in the north and 38^0 43' longitudes in the east at an altitude of 1600 to 2300 m.a.s.l. The district's administrative seat, Meki is found 134 Km away from the capital city, Addis Ababa to the Southeast and 88 Km west along the main asphalt road to Hawasa. The total surface area of the district is 1468 km^2, of which 962.47 km^2 belongs to the total area of land available. The district, which is composed of 36 peasant associations and three urban kebeles, shares border line with Bora Woreda in the North and Northwest, Arsi in the East, ATJK district in the South and Soddo Woreda of SNNPR in the West. In addition, the district is fallen in the Lake Basin of rift valley floor dominated by quaternary sediments which is conducive for farming activities.

Agriculture and land use: According to Dugda district Agricultural and Rural Development Office (2012), the estimated land use pattern data, reveals that 58.40% of the district's land is under cultivation while the rest 1.47%, 10.29%, 12.50% and 0.12% of the land is occupied by forest land, grazing land, water bodies and investment land. On the other hand, 16.91% and 0.31% of the land belongs to settlement and mountains and marsh land, respectively. So, agriculture is the main activities of the district's population and hence it provides the largest livelihood share. Even though there is lack of irrigation facilities, vegetables such as onion, tomato and cabbage are heavily planted and harvested using underground water, Lake Ziway and Meki River, particularly in areas located between Meki town and northern part of Lake Ziway. The general topography of the area slopes towards the east and south and it is characterized by semi-arid climate with an average annual rain fall of about 700 mm. The main soil type in the irrigated field is sandy loam and the mean maximum temperature is about 28 ^{0}C.

Crop production and Water Supply: Crop production of the district is limited to 'Meher' season and the major types of crops that are produced includes maize, wheat, teff, barley and sorghum from cereals, and horse beans, chickpeas and field peas from pulses. Major horticultural crops growing in the irrigated farms around Dugda are Onions, Tomatoes, Cabbage, Papaya and Chili. The district has enough underground water resource potential and currently Meki River

and Lake Ziway have been providing irrigational function under traditional system. The main reason for this endowment is its geographical location for it is situated in between two high lands- the Guraghe highlands in the west and Arsi highlands in the east from where a large amount of surface water drains into the district.

Specific study areas selected for this study are *Walda-Kelina and Shubi-Gemo* agricultural areas located to North-East of Lake Ziway and these areas are selected due to **three** important reasons: **i**. The areas are under continuous cultivation throughout the year and has been supplying significant portion of a wide variety of vegetables like tomato, onion, cabbage, green paper, etc. to the capital city and local people consumption since long time. (**ii**) Modern farming practices such as mechanized farming, application of agrochemicals (fertilizers, pesticides, preservatives, etc), and selected seeds are significant agricultural inputs used for getting better yield in the area. (**iii**) Since these places are found nearby villages, the local community, especially children may be more exposed to these pesticides by eating raw vegetables like tomato as soon as it is sprayed.

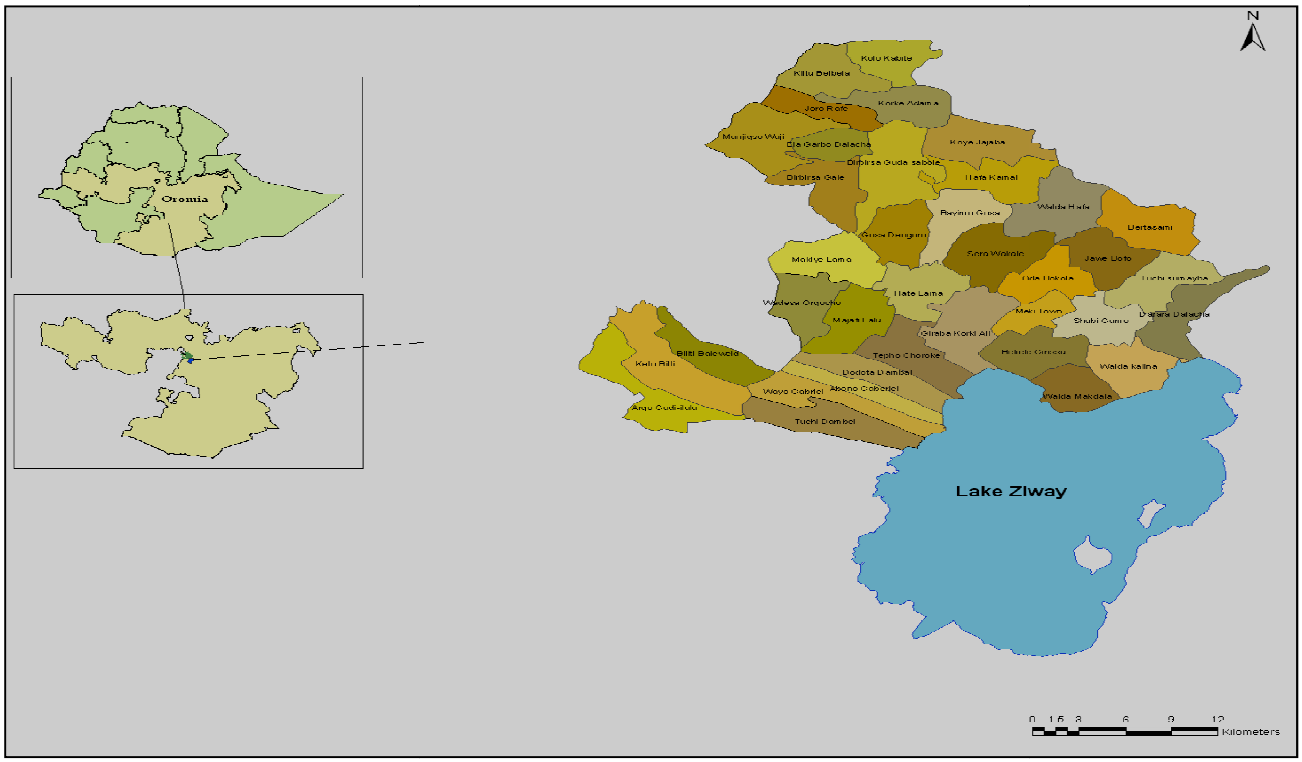


Figure 5: The map of agricultural areas in Dugda district (source: Meki Administrative Office).

3.2. Questionnaire part

3.2.1. Method of data collection

The study used information from the local farmers on some variables such as vegetable production and pesticide use, pesticide application rates, sources and types of pesticides used, farming experiences in these areas, farm size and farm locations.

3.2.1.1. Sampling procedures for the selection of sample kebeles, and respondent farmers

For this study in order to select a representative sample, multi stage sampling techniques were used to select the sample kebeles and house hold respondents. In the first stage, based on the information obtained from Dugda woreda Agricultural Experts and Development Agents, out of 22 rural kebeles of the Dugda Woreda two intensively vegetable producers *Wolda-Kelina* and *Shubi-Gemo* kebeles were selected based on their high level of vegetable production. In the second stage using the house hold list of the sample kebeles, sample farmers were selected and to get the individual farmers for the survey data collection, again a two stage sampling was performed. The first was the selection of the small scale irrigation schemes and this was done on criteria basis.

Accordingly small-scale irrigation users with four and more years of vegetable production experience under small scale irrigation were selected because those farmers would have more exposure to the real situation of irrigated vegetable production and can provide adequate and current information about the types of vegetables growing, pesticide use practice and the rate of pesticide application in those areas. On the second stage to get the final individual respondents systematic random sampling was employed and based on this a total of **80** farmers were selected from both Walda- Kelina and Shubi-Gemo kebeles among **1431** irrigation user farmers.

Data collection and analysis: Both qualitative and quantitative techniques were used for analyzing the data. The quantitative data collected were analyzed using simple description, statistical tools such as mean, percentage, frequency distribution, standard deviation and correlations. The results of the data analysis were presented using tables and graphs as needed and discussions were also made on results.

3.3. Experimental part

3.3.1. Chemicals and Materials

3.3.1.1. Chemicals

I. Pesticide standards (reference materials)

Analytical grades of organochlorine, organophosphorus, and pyrethroid Pesticide standards with their percentage purity: (Chlorpyrifos (99%: China), Diazinone (99.6%: Esrael), Malation (99.6%: Denmark), DDT (99% Israel), Deltamethrine (99%: UK), Endosulphan (alpha+beta = 98.4%: Israel) and Dimethoate (99.2%: Denmark), were donated from Adami Tulu Pesticide processing share company.

II. Organic solvents and reagents

The following **solvents** and **reagents** were used for this study: Ethyl acetate and n-hexane of HPLC grade solvents with percentage purity of 99.9% were obtained from China- SINOCHM company), analytical grade Sodium chloride, anhydrous sodium sulfate (99% Merck extra pure), analytical grade Sodium bicarbonate, $NaHCO_3$ (from India-New Delhi Central Drug House Ltd company), Silica gel adsorbent (mesh > 60%, HPLC grade) and deionized water.

3.3.1.2. Materials

Agilent gas chromatography, model 7980 coupled with FID**,** homogenizer (from Germany-IKAWERKE company), stainless steel commercially heavy–duty blender (from China), high retention Whteman-42 filter paper (size 185 mm from UK GE-Healthcare Ltd company), furnace (from UK-CHESTERFIELD), centrifuge, glass column with 3 cm i.d. and 30 cm length (for silica gel packing), separatory funnel, dropper, magnetic stirrer, chopper, beakers, centrifuge tubes(from Glasco-UK), electronic balance (with precision $\pm$ 0.0001), oven (from England-Wagtech International Ltd company, model 9024055F300), water bath (from England-NICKEL ECTRO Ltd company, model 1104E), crucible, mortar and pestle, conical flasks, spatula, rotary evaporator, gloves and poly ethylene bags (for sampling and storage of field samples) were used.

3.3.2. Sample collection and analysis

In this study, tomato and cabbage samples were collected from two agricultural sites called *Shubi-Gemo and Walda-Kelina* located near the south-west of Lake Ziway. These agricultural areas are primarily known with intense pesticide usage where pesticides are extensively used to enhance the production of vegetables, cereals and fruits, as well as the control of vector born diseases for public health. No studies have been carried out in these agricultural areas despite the fact that pesticides of high toxicity such as endosulphan, deltamethrin, DDT, chlorpyrifos, profenofos (selecron), dichlorovas, diazinone, etc, are used through direct application on the vegetable farms.

The vegetable samples were collected in two different periods because of the intensive cultivation periods and types of pesticides applied in these areas are different for tomato and cabbage. Tomato samples were collected from both areas in the months of January and February in replicate, since these periods are the times of intensive tomato cultivation and high rate of pesticide application to protect the higher pest pressures in these warm times. Since cabbage is cultivated intensively in these areas starting from the month of February, cabbage samples were collected in the months of February and March.

Methods of Sample Collection and preservation: Since these agricultural areas have many small size vegetable farms (one-three hectare), it was necessary to select large and representative vegetable farms (greater or equal to three hectares) to get homogeneous and representative field samples of these vegetables. So, according to FAO guide lines (field sampling technique, 2006) and from information obtained from farmers during field survey, samples were collected based on **farm size**, **types of pesticides frequently applied in these farms** and **the closeness** of the time gap between spray and harvesting times. Based on the information obtained from local vegetable farmers, in these areas tomato and cabbage are commonly harvested and consumed within 3-5 days after the last spray time and when this time range is compared with the minimum half- life that each pesticide has (**Table 1**), it is possible to estimate that there will be higher concentrations of these pesticides in these vegetables above the maximum residue levels set by codex Alimentarium Commission (**Table 10**).

3.3.2.1. Sampling procedure

The sampling procedure and the sampling techniques used in this study were that of the method FAO (2006), (field sampling and preservation methods). **Stratified random sampling** techniques were used in this study by dividing each vegetable farm into ten different strata ('*fila*' according to local names) and then separating and marking by fence. Then from each stratum, **one kilogram sub sample** was collected and then by mixing all these subsamples a total of **ten kilograms composite field samples** were collected for each of tomato and cabbage. So from a single representative farm in a single round, 10 kg composite samples were obtained then these vegetable samples were collected in a clean polyethylene bags, labeled and transported to the laboratory and preserved in a refrigerator at a temperature of 4 ^{0}C before extraction. The method of sample collection, the sampling techniques and ways of sample preservations were similar for both tomato and Cabbage samples except the periods of sample collections for those two kinds of vegetables (tomato from January to February and cabbage from February to March).

3.3.2.2. Extraction of vegetable samples

The method of extraction and clean up used for this study was the USEPA method 3510 (Luke multi residue method*)* for non-fatty types of foods using ethyl acetate and n-hexane as extracting and clean up solvents for organochlorine, organophosphorus and pyrethroid pesticid residues, routinely used by FDA and USDA(Akan *et al*., 2013). These multi residue methods include the steps of preparation, extraction, clean up, chromatographic separations and detections.

3.3.3. Liquid–Liquid Extraction Method (LLE)

Analytes in solutions or liquid samples can be extracted by direct partitioning with an immiscible solvent. Liquid –liquid extraction is based on the relative solubility of an analyte in the two immiscible solvents and is governed by the equilibration distribution/ partitioning coefficient. Extraction of the analyte is achieved by differences in the solubilizing power (polarity) of the two immiscible liquid phases. In this study liquid –liquid extraction techniques were used using ethyl acetate and n-hexane as extracting and clean up solvents.

3.3.3.1. Extraction procedures

Before extraction, each vegetable samples were washed with deionized water and then further categorized by size in to three portions as **large**, **medium** and **small** to get homogeneous samples of each of the cabbage and tomato samples. Then about the same amount were taken from each portion to make a composite sample of 1kg. After washing each of the homogeneous samples for each vegetable with deionized water, they were cut coarsely with stainless steel knife and then homogenized using blender, and for further homogenization, they were placed in a mortar and well ground to paste using pestle.

Then based on USEPA method (for preparation of representative laboratory samples, mostly from 15 g - 50 g), the paste was divided in to two equal portions each with a mass of 30 g (one for spiking) and transferred using spatula to 250 mL conical flasks. Then one of the 30 g of the paste sample was spiked with previously prepared 1mL mixed standard working solutions (mixture of the seven pesticide standards) at a concentration of 0.5µg/mL. Then to each of these samples 100 mL of ethyl acetate and 10 g of sodium chloride (NaCl) were added and shaken vigorously for an hour using a horizontal shaker for the slating out effect. In the same way a reagent blank solution also prepared by adding the same amount of ethyl acetate and treated in the same way as that of the real samples.

After shaking well, 10 g of sodium hydrogen carbonate ($NaHCO_3$) and 40 g of anhydrous sodium sulfate (Na_2SO_4) were added to each and shaken again horizontally for 3 hours and filtered into a beaker. Then after measuring the volumes of all filtrate10 mL of each of the spiked, unspiked and blank samples were centrifuged at 3000 rpm for 5 minutes. Then after separating the top layer, 3 g of Na_2SO_4 was added to each filtrate and shaken, centrifuged at 3000 rpm for another 5 minutes. The aliquots were then evaporated to 5 mL at 40 ^{0}C using rotary evaporator and then reconstituted with 5 mL n-hexane to make it 10 mL. The sodium hydrogen carbonate ($NaHCO_3$) was added to decrease the acidity of the target pesticides in the samples by neutralization so that they can effectively adsorbed on the surface of the acidic silica gel column during clean up stages. The anhydrous sodium sulfate (Na_2SO_4) was added to absorb water from the vegetable matrix (Barlas, 1999).

3.3.4. Cleaning up of vegetable Extracts

All the cleanup procedures, such as **conditioning**, **loading, washing** and **elution** were performed using ethyl acetate plus n-hexane as elution solvents and solid phase extraction column for silica gel packing. **Activating silica gel**: Before packing in to SPE column, the silica gel was activated as follows: 1 kg silica gel was mixed with 2.5 L of deionized water then decanted and dried at 130 ^{0}C for two hours in an oven. Then for further drying and ignition purpose, it was put in a furnace at 660 ^{0}C for 3 hours using crucible. The anhydrous sodium sulfate was also activated by heating in a furnace at 600 ^{0}C for 4 hours (Anastassiades *et al*., 2013).

The vegetable extracts were cleaned up as follows: A 2 cm i.d. with 30 cm length solid phase extracting column was packed with 40 g of activated silica gel in n-hexane and topped up with 3 g of anhydrous sodium sulfate. Then after conditioning the column with 5 mL of n-hexane, the 10 mL extract was added on to the column and the extract was rinsed three times with 2 mL of hexane. Then the pesticide residues were eluted with n-hexane and ethyl acetate according to the following order: **First**, with ethyl acetate: n- hexane (10:90 mL v/v) and **second**, with ethyl acetate: n-hexane (20:80 mL v/v). The cleaned up extracts were then collected and concentrated on a rotary evaporator and dried to 2 mL by water bath. The dried extract then re dissolved in 3 mL n-hexane to have 5 mL final extract, sealed and this was injected and analyzed using GC-FID in the same day.

3.3.5. Preparation of External Standard Solutions

For each target pesticide 1mg/mL stock solutions were prepare by adding 30 mg of each solid standard pesticide in to 30 mL ethyl acetate. Then by diluting each stock solution 10 µg/mL intermediate standard solutions of each pesticide were prepared. Appropriate portions of the intermediate standard solution were again diluted to prepare working solutions of different concentrations for both calibration and spiking. The process was repeated until all the necessary standards concentrations have been prepared. The standard solutions of each pesticide were run on GC-FID under the set of chromatographic conditions and the mean peak areas were plotted against concentrations in ppm of the standards to obtain calibration curves of the individual pesticides.

Calibration curves for each pesticide standard were obtained from running of five point calibration solutions each in triplicate having a concentration range of 0.25µg/mL to 5µg/mL. The lowest concentration level in the calibration curves which was 0.25 µg/mL established as a quantification limit of the instrument for most of the studied pesticides except for three pesticides (DDT, Chlorpyrifos and Endosulfan) which have higher instrumental quantification limit than this value. According to USEPA, (2012), the smallest instrumental quantification limit (IQL) is taken as the spiking amount of a blank for the determination of method detection limit (MDL). Generally the following steps were used for the preparation of stock solution and different working solutions that were used for calibration curves and spiking.

3.3.5.1. Preparation of stock and working solutions for each reference material

Standard stock solutions of each of the studied pesticides were prepared by accurately weighing 30 mg of the solid reference standard and then transferring each to individual 30 mL volumetric flask and then diluting to the volume using ethyl acetate to get 1mg/mL stock solutions. From this stock solution, intermediate solutions of each pesticide were prepared by pipetting 1mL from each and adding into individual 100 mL volumetric flasks and then diluting to the volume with ethyl acetate to get 100 mL of 0.01 mg/mL solution for each reference standard. The primary working solution of each standard was again prepared by pipetting 10 mL of each of the above intermediate solution into 20 mL volumetric flasks and diluting to the volume to get 20 mL of 0.005 mg/mL solution for each. Similarly, through serial dilutions the rest working solutions were prepared to get 0.0025 mg/mL, 0.00125 mg/mL, 0.0005 mg/mL and 0.00025 mg/mL of each standard.

3.3.6. Chromatographic Analysis of Vegetable Samples

All the analytes in this study were determined by gas chromatography coupled with flame ionization detector (GC-FID). The analysis was carried out using Agilent gas chromatography model, 7980 equipped with FID under the following operating conditions: injection temperature, 250 ^{0}c, detector temperature, 300 ^{0}c .Oven temperature program: initial temperature 50 ^{0}c held for 1min and the first ramp temperature 30 ^{0}c/ min to 180 ^{0}c and held for 6 min, the second ramp 1.8 ^{0}c/ min to 230 ^{0}c and held for 10 min, the third ramp 30 ^{0}c/min to 280 ^{0}c and held for 6 min,

finally the fourth ramp 50 ^{0}c/min to 300 ^{0}c for 2 min holding time to give a total run time of 59.17 minutes. Column used for separation was Hp-5 30 m x 320 µm i.d. film thickness 0.25µm with carrier gas nitrogen, purity 99.99%, flow rate 3 mL/min and injection volume of the final extract was 5µL.

Table 2: Apparatus and operating conditions of GC-FID.

Injection types:	Split, 60:1 split ration
Column length and diameter:	30 m length and 320 µm i.d.
Stationary phase:	Hp-5, 5% phenyl, 95% dimethyl poly siloxane
Film thickness :	0.25 µm
Detector:	Flame Ionization Detector (FID)
Detector temperature:	300 ^{0}C
Injector temperature:	250 ^{O}C
Injector liner:	Silanized Pyrex glass
Injection volume:	5µL
Carrier gas:	Nitrogen (N_2): flow rate 3 ml/min.
Make up gas:	Nitrogen(N_2): flow rate 63 ml/min.

Oven temperature programming

	Rate, ^{0}c/min	Initial (temp, ^{o}c)	Holding time (min)	Run time (min)
		50 ^{o}c	1min	1 min
Ramp-1	30	180 ^{o}c	6 min	11.33 min
Ramp-2	1.8	230 ^{o}c	10 min	49.11 min
Ramp-3	30	280 ^{o}c	6 min	56.77 min
Ramp-4	50	300 ^{o}c	2 min	
Total run time				= **59.17 min**

4. RESULTS AND DISCUSSIONS

4.1. Questionnaire part

i. Demographic characteristics of sample house holds

The total sample size of farm respondents handled during the survey was 80 and of the total sample respondents, 92.7% (74) were male headed households and 7.3% (6) were female headed and the average households' age was 37.4 years in both areas. The average year of farming experience related to vegetable production was 7.8 years and this shows that the sampled farmers have good experience in irrigation horticultural farming. The average vegetable farm holding size of the small scale irrigation users was found to be 2.55 hectare per house hold in the study areas and this average household holding of small-scale irrigation land is higher as compared to regional and national average which is 1.5 and 0.95 hectare per household respectively.

Table 3: Land holding per household in Walda-Kelina and Shubi-Gemo agricultural areas.

Hectare	Number of farmers	Percent
0.5-2.5	44	55
2.6-4.3	22	27.5
4.4-5.2	9	11.25
5.3-7.5	4	5
> 7.5	1	1.25
Total	**80**	**100**

Source: Field survey in December 2014

ii. Vegetable production and pesticide use

According to the respondents, the types of vegetable crops mostly growing in the study areas are onion, cabbage, tomato, pepper, papaya, etc and of the above vegetables tomato, cabbage and onion are the very dominant and highly produced commercial vegetables. Most of the farmers (90%) use underground water for irrigation and only 10% of the vegetable growers use Lake

Ziway for irrigation purpose. Family labor is the major source of labor for small scale irrigation farmers and seasonal hired labor is usually employed by house hold with larger vegetable fields.

It is obvious that various studies have proved that appropriate application of modern farm inputs such as improved seeds, pesticides and fertilizers increase agricultural productivity and horticultural crop production predominantly depends on application of those agrochemicals and fertilizers. 95% of the farmers in the study areas use insecticides and fungicides for the protection of vegetables and they get these agrochemicals from the local open market or retailers and the application of pesticides is exercised from the knowledge gained through experience rather than timely training or demonstration processes. Fertility management of the farmer's irrigation plot is performed through chemical fertilizer application and use of animal manure. For vegetables like papaya, the application of manure is done under each individual tree and the effect remains longer and the effective plot is small.

iii. Types of pesticides and their application rates in the study areas

Most of the farmers in the study area use mixtures of liquid and solid (powder) insecticides and fungicides and the frequency or rate of pesticide application is different indifferent seasons of cultivation and according to the respondents higher rate of application of pesticides is necessary in hot rainy summer (june, july and Augest) and 65.7% of the respondents said that they apply a maximum of three times per week during summer season. For example some farmers during the interview said that they usually use Tracer or endosulfan by mixing with mancozeb which is a solid fungicide. They can also use persistent organochlorine insecticides such as DDT to protect tomato and cabbage from insects called white flies mostly in summer season and according to the respondents they bought DDT from the local pesticide sale shops and they told that the price of DDT is low compared with the other organophosphorus and pyrethroid pesticides applied in the area and the price is around 50 birr for 250 g.

Table 4: Types of pesticides, formulations and amount sprayed per hectare for some pesticides used by the farmers in the study areas.

Pesticide	**Formulation**	**Amount per ha/600 L**	**Amount per150 L solution**
Selecron	Liquid	1 L	250 mL
Rogger	Liquid	2 L	500 mL
Malathion	Liquid	2 L	500 mL
Helart	Liquid	2 L	500 mL
Diazinon	Liquid	2 L	500 mL
Dimethoate	Liquid	2 L	500 mL
Korajo	Liquid	60 mL	15 mL
Endosulfan	Liquid	2 L	500 mL
Rodomil	Solid	1kg	0.25 kg
DDT	Solid	2 kg	0.5 kg
Agrolampus	Liquid	1 L	250 mL
Karate	Liquid	0.5 L	125 mL

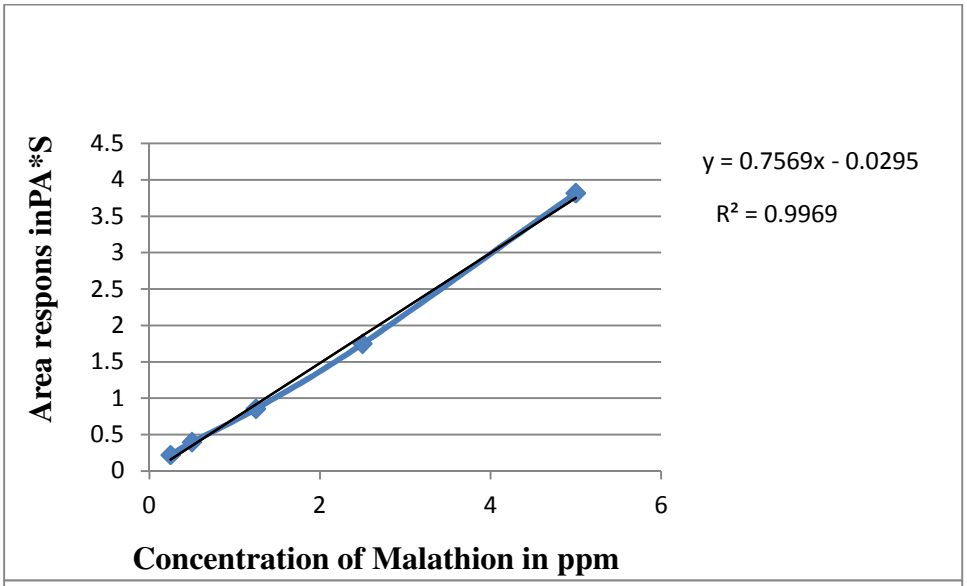
Source: Field survey and from the response of the farmers December, 2014.

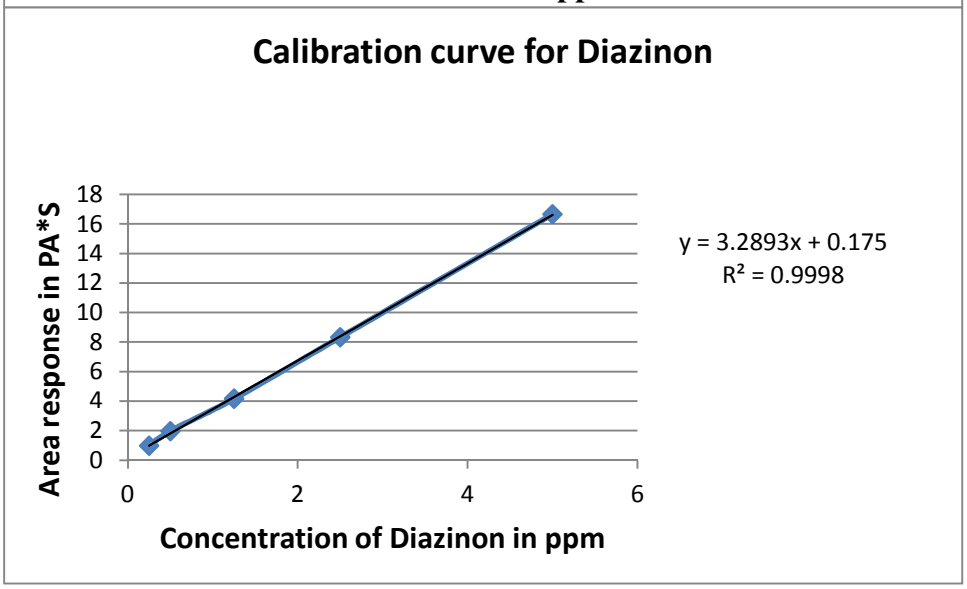
4.2. Experimental part

4.2.1. Method Validation and Quality Control

The study of method validation is one of the quality control measures that should be carried out in residue analysis. To study the validation for quantitative analytical methods it is important to determine some of the following parameters: limit of detection and limit of quantification, precision, accuracy, and percentage recoveries of the samples. In this study the accuracy (percentage recovery), precision (repeatability), limit of detection and limit of quantification were determined for both tomato and cabbage samples.

4.2.1.1. Calibration curves of the standard pesticides for the determination of analytes





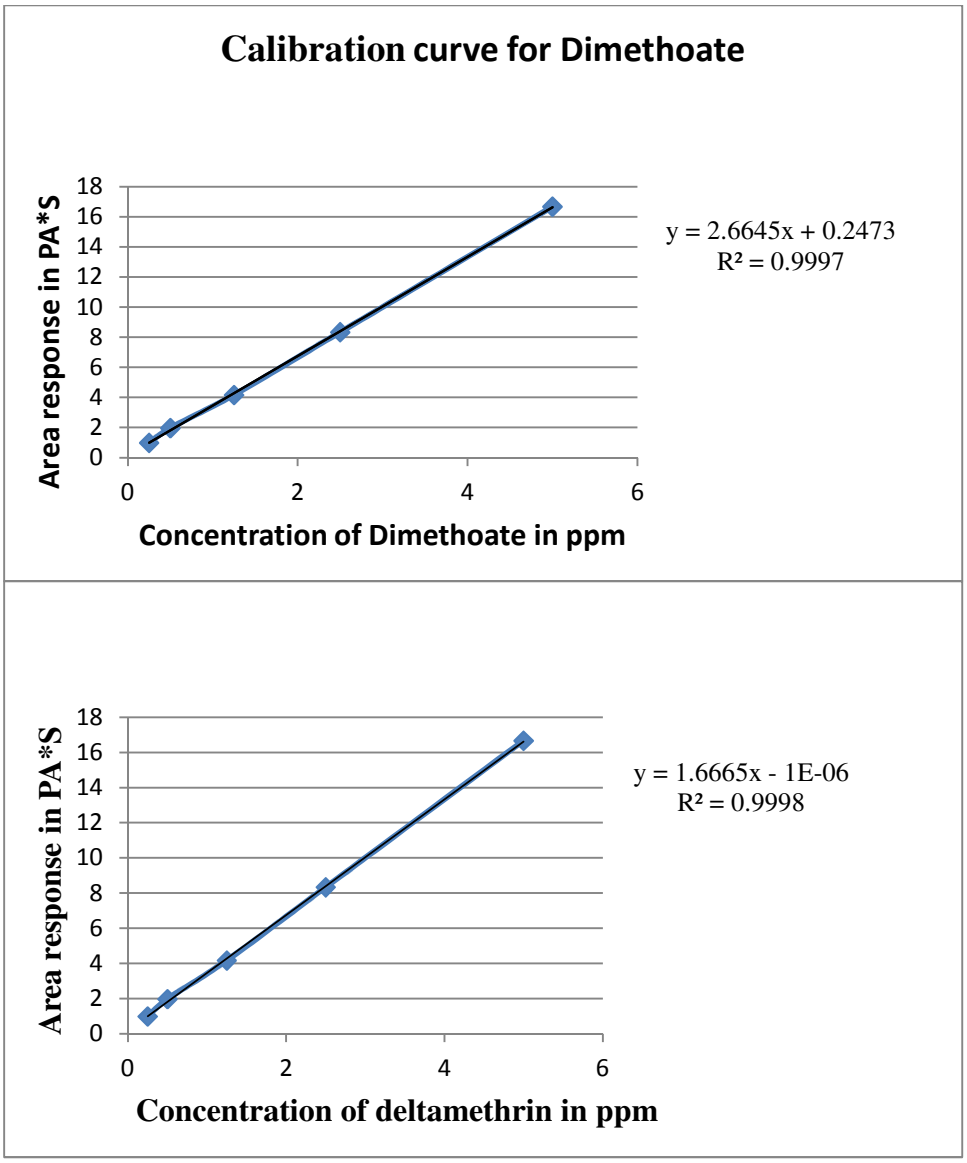


Figure 6: Five point (0.25 ppm to 5 ppm) calibration curves for organophosphorus (Dimethoate, Diazinon, and Malathion) and pyrethroid (Deltamethrine) pesticides standards analyzed by GC-FID.

4.2.1.2. Calibration and Linearity

Calibration was carried out using the above five concentration levels (0.25, 0.5, 1.25, 2.5 and 5) µg/mL for the organophosphorus and pyrethroid pesticide standard solutions specifically for diamethoate, diazinon, malathion and deltamethrine pesticide standards.

In this range the linearity was evaluated based on linear regression and squared correlation coefficients, r^2 which is > 0.999 for all of the pesticide standards except for malathion which 0.9969, slope and y-intercepts of each curve (**Table 5**). The calibration curve for each analyte has a linear range from 0.25-5 µg/mL (**Figure 7**). But the pesticides standards such as chlorpyrifos, DDT and endosulfan have higher instrumental detection limits and show non-linear calibration curves in this range. According to EPA method 3510C, these persistent organochlorine pesticides including the chlorpyrifos are better detected by electron capture detector (ECD) or by GC-MS due to the less sensitivity (high detection limit) of the GC-FID for those pesticides as it is commonly used for volatile organophosphorus pesticides (EPA, 2006). Therefore the non-detect ability of chlorpyrifos, DDT and endosulfan pesticide standards at these lower concentration ranges may be due to having lower vapor pressure (non-volatility) of these highly persistent pesticides (Table1) which is not suitable for this method since the GC-FID method is usually used for highly volatile organophosphorus pesticides such as dimethoate, diazinon, and malathion. So, the above five point calibration points were prepared specifically for the four out of seven pesticides (dimethoate, diazinon, malathion and deltamethrin) and linearity, recoveries and detection limits were evaluated for these pesticides.

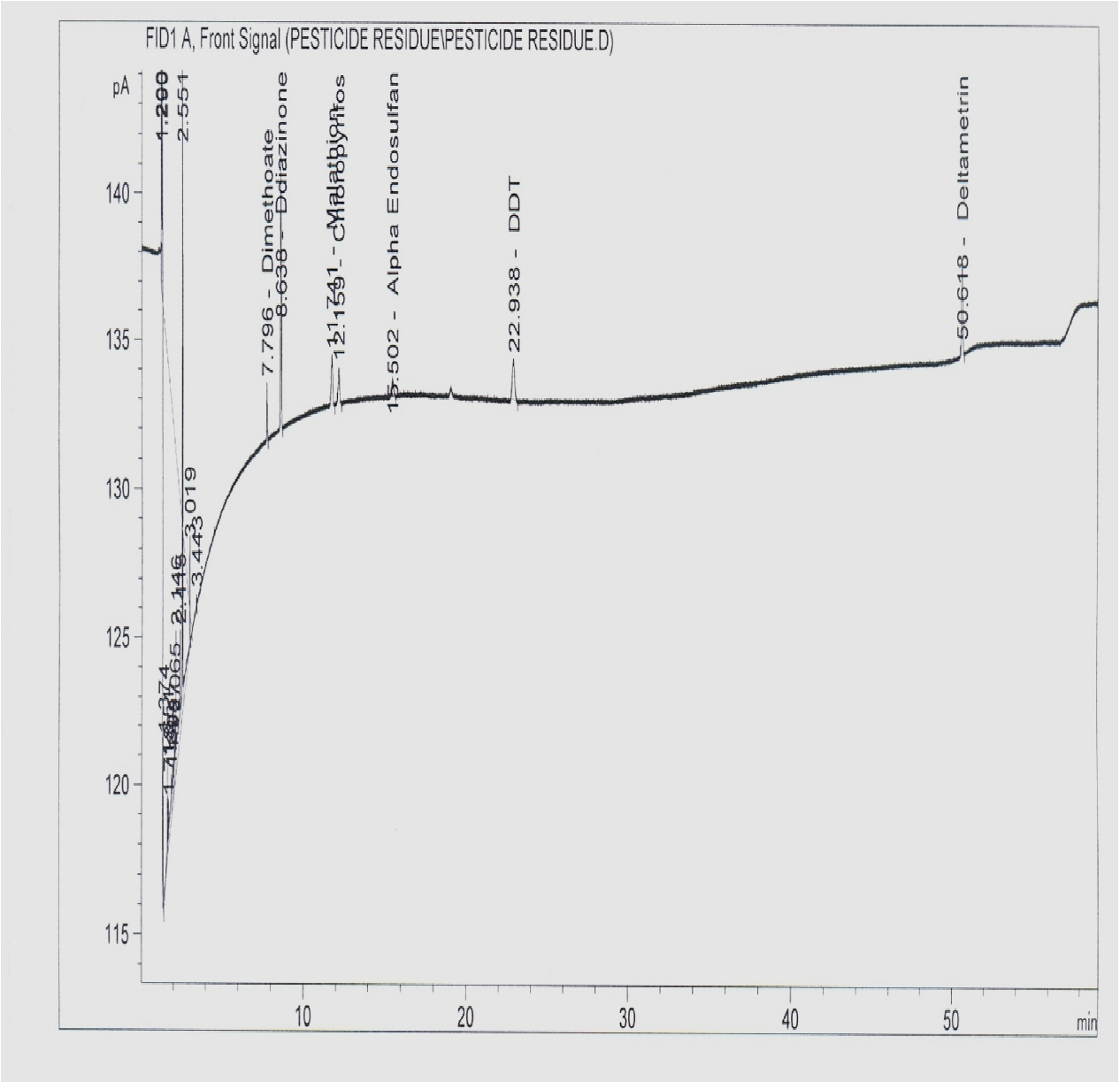


Figure 7: Typical GC-FID chromatogram representation for the complete separation of six pesticides (dimethoate, malathion, diazinon DDT, chlorpyrifos and deltamethrine) in a mixture of standards at 2.5µg/mL

Tables 5: Calibration curve equations, squared correlation coefficient (r^2) and retention times of the studied pesticide standards analyzed by GC-FID.

Pesticides studied	Equations	r^2	Retention time(min)
Dimethoate	y=2.6645X+0.2473	0.9997	7.796
Diazinone	y=3.2893X+0.175	0.9998	8.638
Malathion	y=0.7569X-0.0295	0.9969	11.741
Deltametrin	y=1.6665X-IE-06	0.9998	50.618

Table 6: Peak areas and retention times of the standard pesticides and that of the pesticide residues found in **tomato** and **cabbage** samples.

Pesticide	Pesticide standards at 2 µg/mL		Pesticides found in tomato		Pesticides found in cabbage	
	Peak area	Retention time	Peak area	Retention time	Peak area	Retention time
Dimethoate	0.920823	7.796	0.971148	7.799	0.455930	7.806
Diazinone	6.57067	8.638	4.35958	8.639	1.19092	8.644
Malathion	3.81904	11.741	1.88487	11.752	2.18263	11.753
Deltametrin	2.69263	50.618	7.80828	50.624	13.46145	50.627

4.2.2. Recovery for the method (Accuracy)

To determine the amount recovered for each pesticide spiking method was used by the addition of known quantities of the mixture of the pure standard substances to portions of previously analyzed samples and repeating the analysis using the same reagents, instruments and techniques. The spiking was performed early in the extraction stage and the accuracy of the method was determined by calculating the percentage recovery of each pesticide in each vegetable samples and sample spike (Thompson *et al*., 1999).

The fortification was performed by spiking 2 mL of 0.5µg/mL of the mixed standard solution dimethoate, diazinon, malathion and deltamethrin to 30 g sample before extraction based on the limits of detections and limit of quantification and then extracting the fortified sample by passing through the same process as the pure samples and this spiking level represents value near the instrumental quantification limit on the calibration curves of the pesticides.

The recovery of each pesticide at this fortification level was calculated by the following formula and the percent recoveries of each pesticide in both tomato and cabbage were presented below (Table 7).

$$\%R = \frac{C\ spike - C\ sample}{Spiked\ Conc} X\ 100 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

C sample = unspiked sample in µg/g,

C spike = the result of the spiked in µg/g Spiked conc. = 0.5 µg/mL

%R=the amount of pesticide recovered in percent.

According to European commission recommendation mean recoveries from 70-100% are satisfactory (Karakas, 2006). From the recovery data presented in Table 9, for spiked tomato sample, the percentage recovery of diazinon is below the acceptable range and for chlorpyrifos it is in the recommended range and that of the DDT is good percent recovery. Endosulfan was not detected at this fortification level. From the mean recoveries of the analytes in the cabbage sample spike, deltamethrin and chlorpyrifos have percent recoveries in the acceptable range but that of malathion dimethoate and diazinon were lower. DDT and endosulfan were not detected in the cabbage spike at this fortification level.

The lower percent recoveries of diazinon, malathion, and dimethoate may be due to more volatile nature of those pesticides than the others and may escaped during the extraction and storage process or they may be highly adsorbed on the adsorbent of the clean up column during the clean up stage. DDT and endosulfan were not detected in cabbage spiked sample may be due to the higher detection limits of these pesticides above the fortification level for this method and to detect these pesticides at this fortification level other gas chromatography detectors such as ECD and MS may be used. Similarly endosulfan was not detected in the spiked tomato sample may be because of lack of these appropriate detectors for organochlorine pesticides.

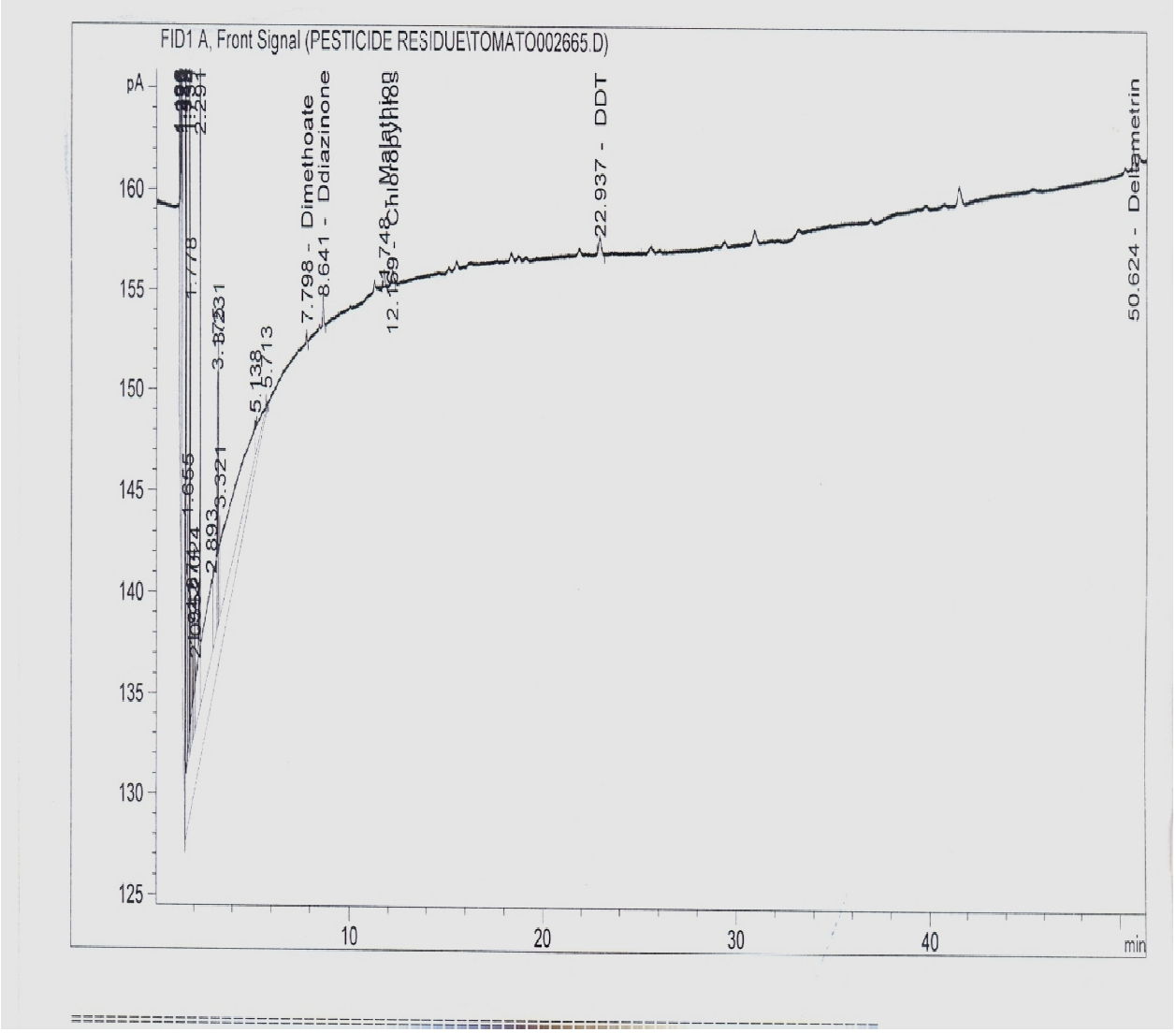


Figure 8: Chromatogram for **tomato sample** spiked at 0.5 µg/mL of a mixed standard solution and extracted by LLE method and analyzed by GC-FID.

Table 7: Spiked amount (0.5µg/mL), calculated mean concentrations of pesticides in the spiked samples and the percent recoveries of tomato and cabbage spiked samples.

Pesticides	**Spiked level (µg/ml)**	**Peak area of tomato spike**	**Peak area of cabbage spike**	**Calcu. mean conc. (Spike + sample) (mg/kg)**		**Mean recovery (R) (%)**	
				Tomato	**Cabbage**	**Tomato**	**Cabbage**
Dimethoate	0.5	1.58033	0.591698	0.57	0.21	44	40
Diazinone	0.5	6.49849	4.41269	0.33	0.23	22	34
Malathion	0.5	5.09589	2.93795	0.45	0.26	58	14
Chlorpyrifos	0.5	4.28986	3.01903	0.50	0.35	100	70
Endosulfan	0.5	ND	ND	ND	ND	ND	ND
DDT	0.5	7.50295	ND	0.61	ND	122	ND
Deltamethrin	0.5	5.90064	5.58477	0.73	0.69	48	98

ND = Not Detected

4.2.3. Precision of the method

In the study the repeatability of the method was studied by running five extractions which were spiked at 0.5 µg/mL each with the mixture of dimethoate, diazinon, malathion, and deltamethrine pesticides and extracted within a day. The standard deviation and the relative standard deviation values for each pesticide were determined using the following equations and the spiked amount, the calculated concentrations, standard deviation and relative standard deviations are presented in Table 10 for tomato spike and Table11 cabbage spike below.

$$SD = \sqrt{\frac{[\sum(X - X1)2 + \cdots + (X - Xn)2]}{n - 1}} \quad \text{.....(2}$$

$$RSD\ (\%)\ \frac{SD}{X}.\ 100 \quad \text{.....(3)}$$

Table 8: Determination of the repeatability of the method for the studied pesticides using spiked **Tomato sample** at 0.5 µg/mL with pesticide mixtures and extracted by ethyl acetate: n-hexane (90:10 v/v) and (80:20 v/v).

pesticides	Spiked amount (µg/mL)	Calculated mean concentration(µg/g)	SD (µg/g)	RSD	Mean(x)±SD
Dimethoate	0. 5	0.42	0.022	5.2	0.42±0.022
Diazinon	0. 5	0.35	0.041	11.7	0.35±0.041
Malathion	0. 5	0. 47	0.015	3.19	0.47±0.015
Deltamethrin	0. 5	0.49	0.0103	2.1	0.49±0.0103

n=5

Table 9: Determination of the repeatability of the method for the studied pesticides using spiked **Cabbage sample** at 0.5 µg/mL with pesticide mixtures and extracted by ethyl acetate: n-hexane (90:10 v/v) and (80:20 v/v).

pesticide	**spiked amount (µg/mL)**	**Calculated mean concentration(µg/g)**	**SD (µg/g)**	**RSD**	**Mean(x) ±SD (µg/g)**
dimethoate	0.5	0.31	0.071	7.1	0.31±0.071
Diazinon	0.5	0.43	0.044	4.4	0.43±0.044
Malathion	0.5	0.44	0.040	4.0	0.44±0.0400
Deltamethrin	0.5	0.37	0.063	6.3	0.37±0.063

n=5

From Tables 8 and 9 above, the percent relative standard deviation values of the pesticides in both tomato and cabbage are not more than 11.7% and found in the acceptable range, therefore this indicates that the method used is repeatable under the available laboratory condition.

According to the Food Safety and Standard Authority of India, (2012), the residue level of each pesticide from vegetable samples can be calculated by the formula:

$$R = \frac{\text{peak area of the sample}}{peak\ area\ of\ the\ standard} \frac{\mu L\ of\ the\ standard\ injectd}{\mu L\ of\ the\ sample\ injected} \text{X} \frac{\text{extracted volume}}{mass\ of\ the\ sample} \text{xppm of reference}$$

Mass of the sample = 30 g, Extracted volume = 5 mL

R= Residual level of the pesticide (µg/g) Volume of the standard injected = 5µL

ppm of the reference = 2 ppm Volume of the sample injected = 5µL

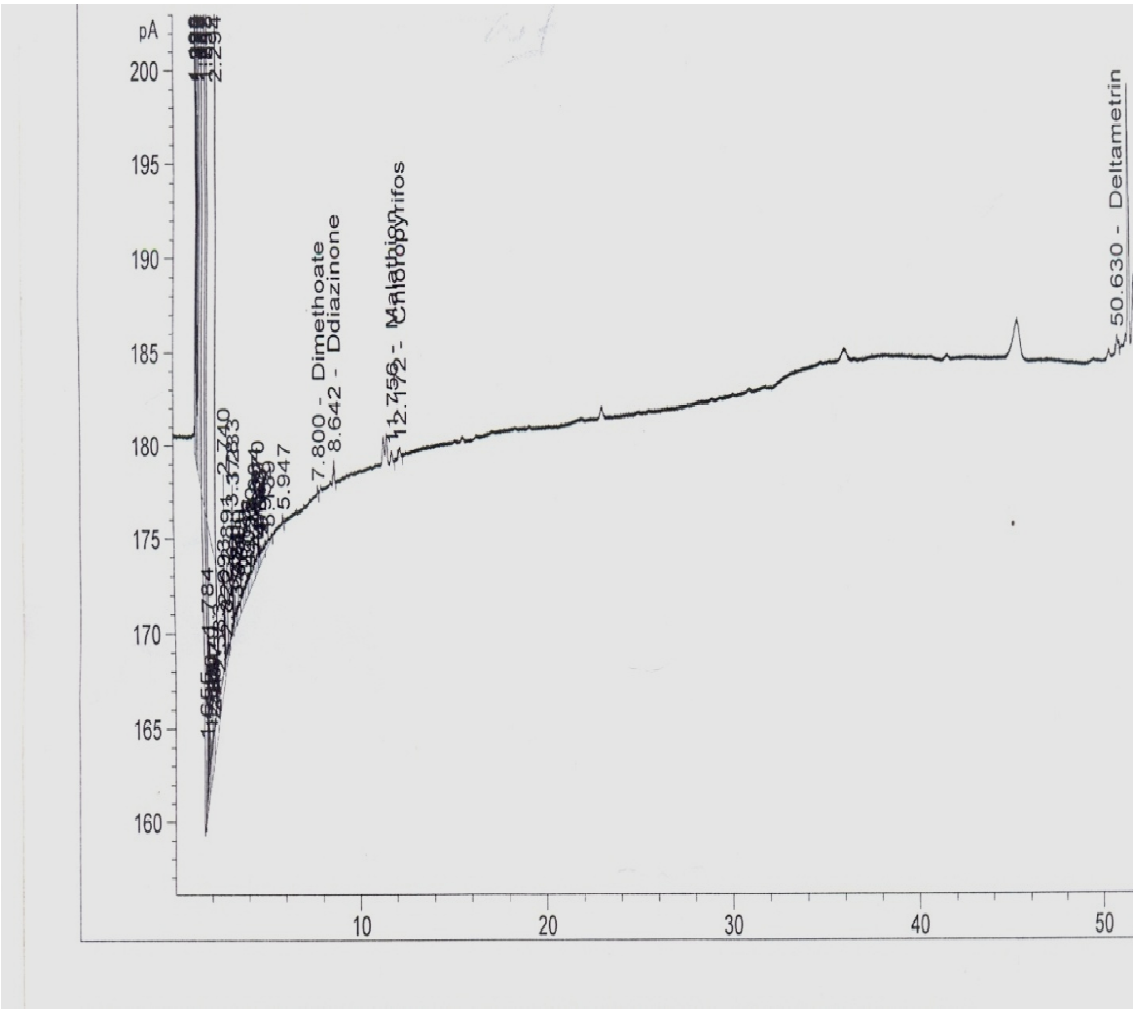


Figure 9: Chromatogram for Tomato sample, extracted by LLE and analyzed by GC-FID

4.2.4. Limit of detection and limit of quantification

The method detection limit for each pesticide was determined by spiking a blank sample at 0.25 µg/ml of a mixture of pesticide standards that contains each pesticide standard and extracting the spiked blank sample by the same procedure as the pure sample and then analyzing the mixture after five replicate injections into the GC-FID. The means and standard deviations of the results of the five replicate injections for each pesticide were calculated. At this fortification level DDT, endosulfan, and chlorpyrifos and were not detected due to higher instrumental detection limits for these pesticides and it is important to prepare a higher spiked level. The quantification limit was determined as three time of the method detection limit

$$MDL = 3\ S_B \quad \ldots\ldots (5)$$

$$LOQ = 10\ S_B = 3MDL \quad \ldots\ldots (6)$$

S_B = the standard deviation of the response

MDL= Method detection limit

LOQ = Limit of quantification = 0.25 µg /mL

Table 10: Residual concentrations of each pesticide in **cabbage** and **tomato** samples with international maximum residue limit (MRL) *and* detection limits.

Pesticides	**Concentration (µg/g)**		**MRL(µg/g)**		**Blank spike level (µg/ml**	**Tomato**		**Cabbage**	
	Tomato	**Cabbage**	**Tomato**	**Cabbage**		**LOD (µg/g**	**LOQ (µg/g**	**LOD (µg/g)**	**LOQ (µg/g)**
Dimethoate	0.35	0.15	1.0	2.0	0.25	0.10	0.31	0.06	0.18
Malathion	0.16	0.19	3.0	8.0	0.25	0.08	0.22	0.03	0.09
Diazinon	0.22	0.06	0.61	2.0	0.25	0.04	0.12	0.06	0.18
Endosulfan	ND	ND	0.5	1.0	0.25	ND	ND	ND	ND
Chlorpyrifs	ND	ND	0.3	0.05	0.25	ND	ND	ND	ND
DDT	ND	ND	-	-	0.25	ND	ND	ND	ND
Deltamethrin	0.97	1.67	0.3	0.5	0.25	0.03	0.08	0.02	0.06

ND= non detected

Source for MRL: Joint FAO/WHO Food Standard Program Codex Alimentarium Commission, Geneva, Switzerland, July, 2014.

From the above parameters we can observe that dimethoate, malathion, diazinon and deltamethrin pesticides have residual concentrations of 0.35, 0.16, 0.22 and 0.97 µg/g in tomato sample respectively and the residual concentrations of the same pesticides in cabbage sample respectively are 0.15, 0.19, 0.06 and 1.67 µg/g. When we compare these residual concentrations found in both tomato and cabbage with the maximum residue limit, they are lower except for deltamethrin which is found at higher residual concentrations than the MRL in both tomato and cabbage samples. The lower residual concentrations of dimethoate, diazinon, malathion than MRL may indicate that there might be efficient way of using these pesticides for tomato and cabbage.

But the higher residual concentrations of deltamethrin in both tomato and cabbage than the MRL may be due to there might be improper usage or high rate of applications of deltamethrin near the harvesting times of these vegetables or it might be because of the high persistent nature of deltamethrin than organophosphorus pesticides. Again from the above table we can see that three pesticides (endosulfan, DDT and chlorpyrifos are not detected in both tomato and cabbage samples. The non-detectable concentrations of endosulfan, DDT and chlorpyrifos pesticides in both cabbage and tomato samples may be due to either lack of

proper method of extraction or may be due to low sensitivity of GC-FID for those relatively persistent pesticides rather it is mostly used for the analysis of volatile pesticides from these kinds of vegetables. So the extraction, clean up and analysis methods used in this study are more efficient for organophosphorus and pyrethroid pesticides rather than the organochlorines such as DDT and endosulfan including chlorpyrifos.

The limit of detections of all of the detected pesticides (dimethoate, Malathion, diazinon and deltamethrin) are below the MRL, set by codex (2009) and this shows that this method is more applicable for detecting the oranophosphorus and pyrethroid pesticides effectively even at concentration lower than the maximum residue limit. In the analysis of blank samples spiked at 0.25 µg/mL concentration, endosulfan, DDT and chlorpyrifos are not detected in all injections for both tomato and cabbage and this indicates that the limit of detection of these pesticides by this method are higher than 0.25µg/mL and in order to detect this level of concentration for these pesticides it better to use more selective detectors like ECD and MS.

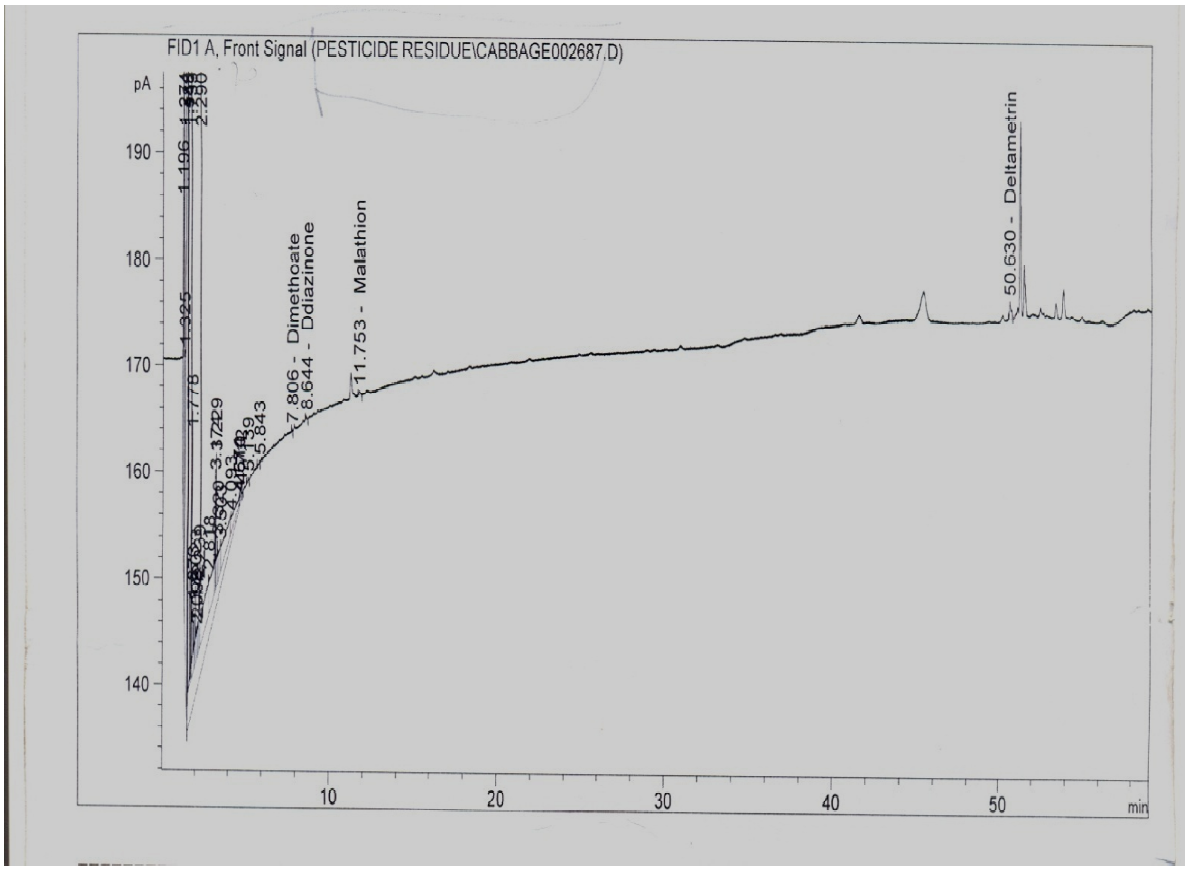


Figure 10: Chromatogram for cabbage sample extracted by LLE and analyzed by GC-FID

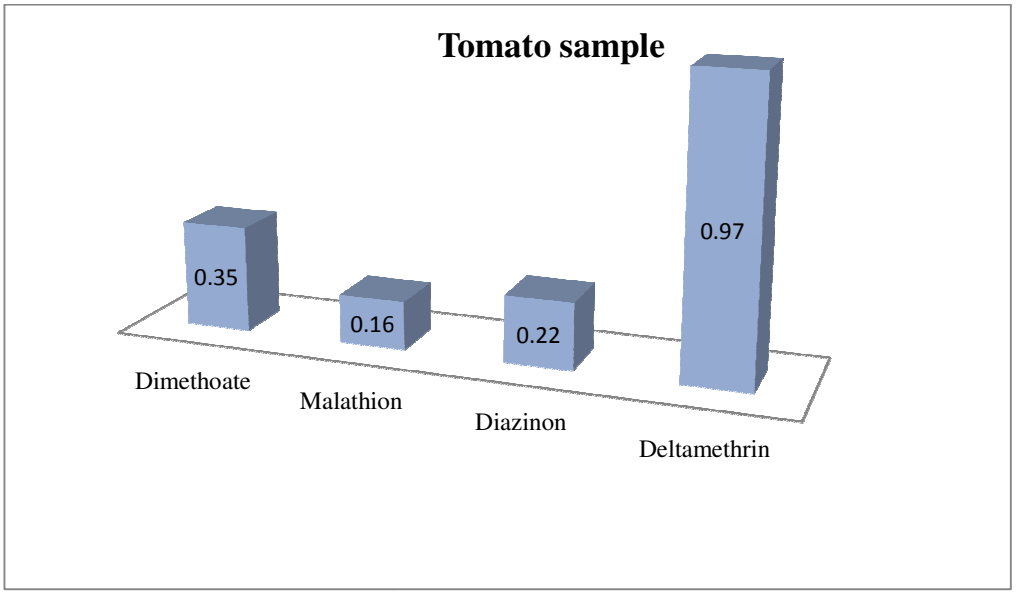


Figure11: Representation of the residual concentrations of dimethoate,Malathion, diazinone, and deltamethrin in tomato sample.

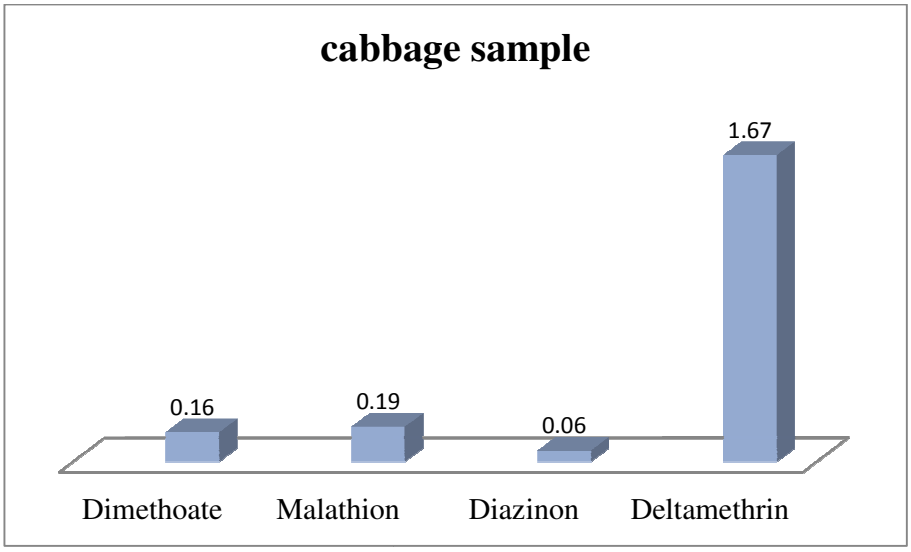


Figure 12: representation of the residual concentration of Dimethoate, Malathion, Diazinon and Deltamethrin in Cabbage sample

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSIONS

The results of the analysis have indicated that these fresh tomato and cabbage collected from Dugda Woreda agricultural sites are contaminated at least with three organophosphorus (dimethoate diazinon, malathion) and one pyrethroid pesticide (deltamethrin). From the calibration curves of these four pesticides we can see that they are detectable at lower residual concentrations and all have detection limits that are lower than the MRL which shows that the residual concentrations of these pesticides are detectable even below this maximum residue limit by this method. The residual concentrations of deltamethrin in both tomato and cabbage are above the maximum residue limit set by Codex Alimmentarium Commission.

The result has also indicated that organochlorine pesticides such as endosulfan and DDT were not detected in both tomato and cabbage samples. From the method validation parameters such as limits of detection and quantification we can also generalize that the method used for this study is not suitable for the quantification of these organochlorine pesticides. But the result obtained from the interviewed farmers during the field survey indicated that even though these pesticides were not quantitatively detected in these vegetables by this method, farmers in the study areas are using these persistent pollutants in both vegetables (Table 6).

Generally from both the analysis and information obtained from respondent farmers in the study areas, unless farmers use other pest control methods such as IPM, the contamination of vegetables is an ongoing process and pesticide residue in vegetables may be unavoidable even when they are used in accordance with good agricultural practices. That is why in these research, the main purposes are determination of these pollutants in tomato and cabbage and comparing their residue amount with the maximum residue limit set by Codex Alimentarium Commission. So it is clear that the consumption foods containing unsafe amount of pesticide residues are of public health concern and affect economic development by affecting the export quality of vegetables.

5.2. RECOMMENDATIONS

Consumers usually perceive the damaged or decayed vegetables as low quality products and they think that fresh fruits and vegetables are safe and free of contaminants. But most of the time chemical contaminants are not perceived either by observation or tasting. Similarly in this study it was observed that fresh tomatoes and cabbages which were not decayed and look healthy were however contaminated with organophosphorus and pyrethroid pesticides. Therefore, from the results of this research together with the ideas obtained from respondent farmers, the following issues should be raised and described as follows.

- Farmers should be trained on how to spray and on rates of applications of pesticides especially near the harvesting periods.
- Workers and children that live nearby these farming areas should avoid eating uncooked vegetables after spraying.
- Further studies should be carried out to know the safe time gap between harvesting and application of pesticides.
- Other alternative pest protection methods such as biological control and integrated pest management systems should be adopted rather than totally relay on pesticide.

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Annex I

Questionnaire designed to assess **the pesticide use and application rates**, **types of vegetables growing in the area, types of common pests of vegetables and pest control mechanisms** by small scale irrigation farmers in *Shubi-Gemo* and *Walda-Kelina* agricultural sites of Dugda Woreda, East Showa Zone**.**

Date _____

Time _____

Name of data collector_____ Signature____

Name of the supervisor_____ Signature____

1. Introduction

My name is ---------- and I am a student who is carrying out a study on the residue levels of pesticides commonly sprayed on tomato and cabbage vegetables in your area.

I would very much appreciate your participation in this survey and I would like to ask you some questions related to pesticides which will take you only 15 minutes. Your answer will remain confidential and I will not be taking part down your name or address, so your answer will be anonymous. Participation in this survey is voluntarily and you can choose not to answer any individual questions, however, I hope that you will participate in this study since your views are important. At this time do you want to ask me anything about the survey?

May I start asking you the survey questions?

General Information

I. Back ground to the house hold and farm

1. Woreda ---------- Kebele ----------
2. Number of the respondent ---------
3. Age: i. 15- 25 ii. 25-35 iii. 35-45 iv. 45-55 v. above 55
4. Sex: i. Male ii. Female
5. Are you the head of the family? i. Yes ii. No
6. Level of Education: i. none ii. Grade 7-8 iii. Grade 9-10 iv. Grade 11-12 v. higher edu.
7. Marital status: i. Single ii. Married iii. Divorced
8. Experience in Farming (in years): i. < 3 yrs ii. 3-5 yrs iii. 5-7 yrs iv. > 7 yrs
9. Farm size (in hectare): i. <1 ha ii. 1-3 ha iii. 3-4 ha iv. 4-5 ha v. > 5 ha
10. Farm location: i. In hills ii. Near water iii. Wetland areas iv. Near villages

I. Vegetable production and pesticide use

1. What types of vegetables do you grow and for what purpose?

		Subsistence	Commercial	Source of water	
				Underground	Surface
i	Tomato				
ii	Cabbage				
iii	Onion				
iv	Green paper				
v	Others(please specify)				

2. What types of pesticides do you use for?

	Tomato	Cabbage	Onion	Green paper
Types of pesticides used	1. 2. 3. 4. 5.	1. 2. 3. 4. 5.	1. 2. 3. 4. 5.	1. 2. 3. 4. 5.

3. For what purpose do you use them?	1. Control of insects
	2. Control of weeds
	3. Control of fungi molds/rusts
	4. Control of rodents
	5. Others (please specify)

Iv. Pesticide application rate and safety rules

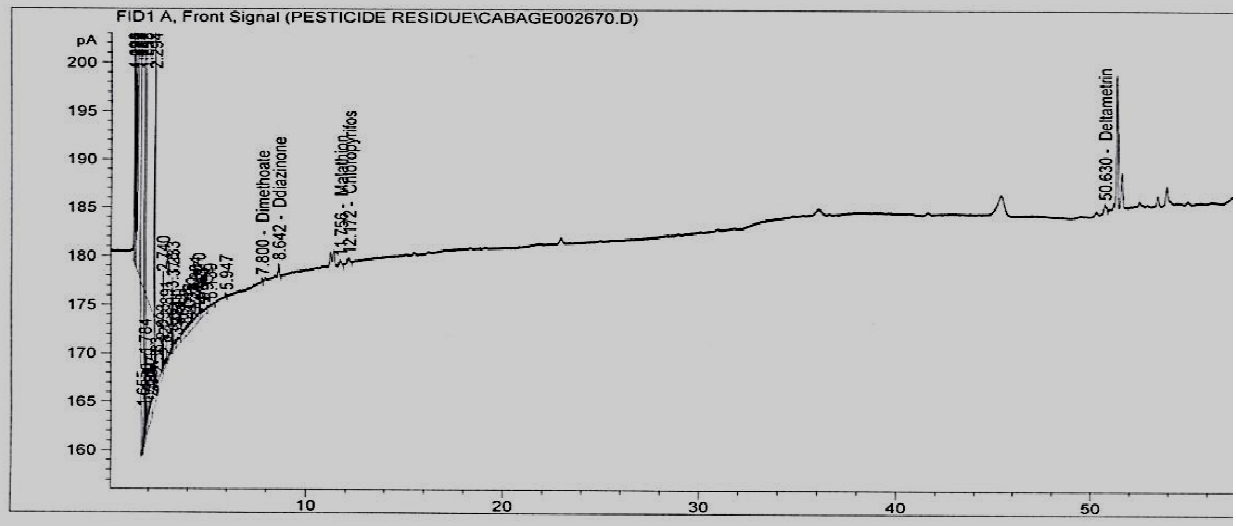
1. When (season/month) do you apply pesticides mostly?	
2. How many times do you apply each pesticide (per week)?	i. One time ii. Two times iii. Three times iv. Four times
3. When do you harvest the vegetables after your last spray?	i. 3-5 days ii. 5-7 days iii. >7 days
4. What size /area are the fields you apply pesticide to?	
5. Who apply them?	
6. What do you wear when you apply pesticides?	i. Gloves, ii. Eye-glass, iii. Tuta, iv. Nosal wear, v. Others
7. Have you had training about use of pesticides?	i. Yes ii. No
8. If 'yes', what were you trained on?	
9. Do you usually read the labels on pesticides containers?	i. Yes ii. No
10. Have you ever used chemical with instructions in a language you do not understand?	i. Yes ii. No
11. From where do you get the pesticides?	i. Local market ii. Cooperatives iii. Research centers
12. What do you do with empty pesticide containers?	
13. Does your pesticide use solve your pest problems?	i. Yes ii. No

Annex II

The Results of the Pesticide Analysis in Tomato and Cabbage Samples using Gas Chromatography Flame Ionization Detector (GC-FID)

Data File C:\CHEM32\1\DATA\PESTICIDE RESIDUE\CABAGE002670.D
Sample Name: 23-04-15 cabage spike

```
Acq. Operator    : gemechu
Acq. Instrument  : Instrument 1                    Location : Vial 4
Injection Date   : 23-Apr-15 1:32:18 PM
                                                   Inj Volume : 5 µl
Acq. Method      : C:\CHEM32\1\METHODS\PESTICIDE RESIDUE2.M
Last changed     : 23-Apr-15 1:25:28 PM by gemechu
                   (modified after loading)
Analysis Method  : C:\CHEM32\1\METHODS\PESTICIDE RESIDUE2.M
Last changed     : 23-Apr-15 2:31:30 PM by gemechu
                   (modified after loading)
Method Info      : vegitable pesticide residue
```




```
                        Internal Standard Report

Sorted By             :      Signal
Calib. Data Modified  :      19-Apr-15 8:39:04 AM
Multiplier            :      1.9000
Dilution              :      1.0000
Sample Amount:        :      1.00000  [%]   (not used in calc.)
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal
```

RetTime [min]	Type	ISTD used	Area [pA*s]	Amt/Area ratio	Amount [%]	Grp	Name
7.800	BBA	+	5.91698e-1	0.00000	0.00000		Dimethoate
8.642	BB	+	4.41269	0.00000	0.00000		Ddiazinone
11.756	BB	+	2.93795	0.00000	0.00000		Malathion
12.172	BB	+	3.01903	0.00000	0.00000		Chloropyrifos
15.518			-	-	-		Alpha Endosulfan

Data File C:\CHEM32\1\DATA\PESTICIDE RESIDUE\CAHA0R002670.D
Sample Name: 23-04-15 cabage spike

RetTime [min]	Type	ISTD used	Area [pA*s]	Amt/Area ratio	Amount [%]	Grp	Name
18.232			-	-	-		propoxure
19.092			-	-	-		Beta Endosulfan
22.965			-	-	-		DDT
50.630	MM	+	5.58477	0.00000	0.00000		Deltamethrin

Totals without ISTD(s) : 0.00000

12 Warnings or Errors (10 first messages follow) :

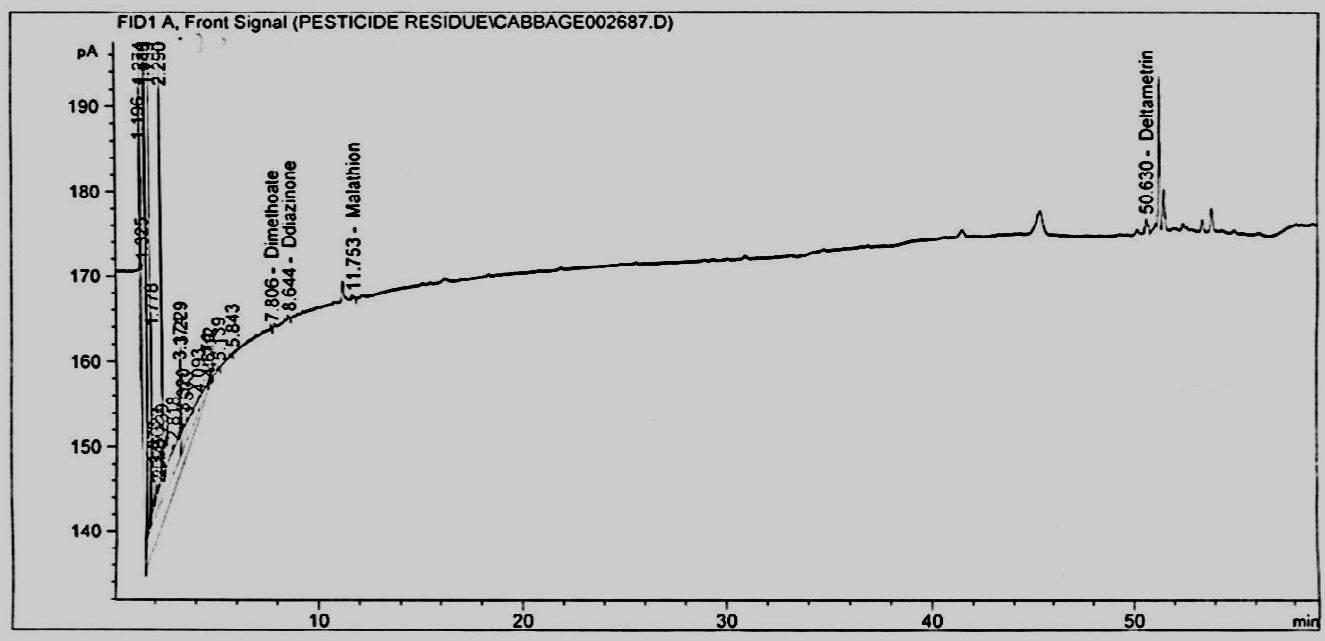
Warning : Calibration invalid (see calibration table listing)
Warning : Time reference compound(s) not found
Warning : ISTD compound missing, (Dimethoate)
Warning : Invalid calibration curve, (Dimethoate)
Warning : ISTD compound missing, (Ddiazinone)
Warning : Invalid calibration curve, (Ddiazinone)
Warning : ISTD compound missing, (Malathion)
Warning : Invalid calibration curve, (Malathion)
Warning : ISTD compound missing, (Chlorepyrifos)
Warning : Invalid calibration curve, (Chloropyrifos)

==========

*** End of Report ***

Data File C:\CHEM32\1\DATA\PESTICIDE RESIDUE\CABBAGE002687.D
Sample Name: 24-04-15 cabbage sample

Acq. Operator : gemechu
Acq. Instrument : Instrument 1 Location : Vial 6
Injection Date : 24-Apr-15 10:43:07 AM
Inj Volume : 5 µl
Acq. Method : C:\CHEM32\1\METHODS\PESTICIDE RESIDUE2.M
Last changed : 23-Apr-15 2:46:14 PM by gemechu
Analysis Method : C:\CHEM32\1\METHODS\PESTICIDE RESIDUE2.M
Last changed : 24-Apr-15 11:42:19 AM by gemechu
Method Info : vegitable pesticide residue



Internal Standard Report

Sorted By : Signal
Calib. Data Modified : 19-Apr-15 8:39:04 AM
Multiplier : 1.9000
Dilution : 1.0000
Sample Amount: : 1.00000 [%] (not used in calc.)
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: FID1 A, Front Signal

RetTime [min]	Type	ISTD used	Area [pA*s]	Amt/Area ratio	Amount [%]	Grp	Name
7.806	BBA	+	4.55930e-1	0.00000	0.00000		Dimethoate
8.644	BB	+	1.19092	0.00000	0.00000		Ddiazinone
11.753	BB	+	2.18263	0.00000	0.00000		Malathion
12.174			-	-	-		Chloropyrifos
15.518			-	-	-		Alpha Endosulfan
18.232			-	-	-		propoxure
19.092			-	-	-		Beta Endosulfan

Data File C:\CHEM32\1\DATA\PESTICIDE RESIDUE\CABBAGE002687.D
Sample Name: 24-04-15 cabbage sample

RetTime [min]	Type	ISTD used	Area [pA*s]	Amt/Area ratio	Amount [%]	Grp	Name
22.965			-	-	-		DDT
50.630	BBA	+	13.49518	0.00000	0.00000		Deltametrin

Totals without ISTD(s) : 0.00000

10 Warnings or Errors :

Warning : Calibration invalid (see calibration table listing)
Warning : Time reference compound(s) not found
Warning : ISTD compound missing, (Dimethoate)
Warning : Invalid calibration curve, (Dimethoate)
Warning : ISTD compound missing, (Ddiazinone)
Warning : Invalid calibration curve, (Ddiazinone)
Warning : ISTD compound missing, (Malathion)
Warning : Invalid calibration curve, (Malathion)
Warning : ISTD compound missing, (Deltametrin)
Warning : Invalid calibration curve, (Deltametrin)

*** End of Report ***