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DETERMINATION OF ORGANOCHLORINE AND ORGANOPHOSPHORUS PESTICIDE RESIDUES IN TOMATOES, POTATOES AND PINEAPPLES FROM SELECTED FARMLANDS IN SOUTHWEST ETHIOPIA

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MSC THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES COLLEGE OF NATURAL SCIENCE JIMMA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY (ANALYTICAL).

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ABBREVIATIONS AND ACRONYMS

AChE .....Acetyl Cholinesterase Enzyme

AOAC.....Association of Official Analytical Chemists

AR.....Analytical Reagent

AIDS.....Acquired Immune Deficiency Syndrome

ANOVA .....Analysis of Variance

CAC .....Codex Alimentarius Commission

ChE .....Cholinesterase Enzyme

CNS.....Central Nervous System

CV.....Coefficient of Variation

DBC.....Dibutyl Chlorinedate

DDE.....Dichlorodiphenyldichloroethylene

DDT.....Dichlorodiphenyltrichloroethane

d-SPE.....Dispersive Solid Phase Extraction

EIAR.....Ethiopian Institute of Agricultural Research

EU.....European Union

FAO.....Food and Agricultural Organization

GAP.....Good Agricultural Practices

GDP.....Gross Domestic Product

GC----------.Gas Chromatography

GCB.....Graphitized Carbon Black

GC-ECD.....Gas Chromatography-Electron Capture Detector

GC - NPD.....Gas Chromatography Nitrogen Phosphorus Detector

GC - FPD.....Gas Chromatography-Flame Photometry Detector

HPLC----------...High-Performance Liquid Chromatography

IPM.....Integrated Pest Management

JARC.....Jimma Agriculture Research Center

LOD.....Limit of Detection

LOQ .....Limit of Quantification

LSD .....Least Significance Difference

LDR.....Linear Dynamic Range

LC-MS/MS.....Liquid Chromatography-tandem Mass Spectrometry

MRMs.....Multi-Residue Methods

MRL.....Maximum Residues Limit

MS/MS.....Tandem Mass Spectrometry

MS.....Mass Spectrometry

OCPs .....Organochlorine Pesticides

OPPs.....Organophosphorus Pesticides

OPIDP .....Organophosphate-Induced Delayed Polyneuropathy

PSA.....Primary and Secondary Amine

PLC.....Private Limited Company

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

RSD.....Relative Standard Deviation

RCBD.....Randomized Complete Block Design

SAS.....Statistical Analysis Software

SD.....Standard Deviation

SPE.....Solid Phase Extraction

WHO.....World Health Organization

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ABSTRACT

Pesticides are widely used in Ethiopian agriculture to increase the yield, improve the quality, and extend the storage life of horticultural crops. But wide uses of these chemicals could result in contamination of air, water, food, and ecosystem. In this study, determination of organophosphates and organochlorines pesticides residues in tomato, potato and pineapple samples from selected farmlands of Jimma zone and Kefa zone, in the southwest of Ethiopia was undertaken. The samples were collected from tomato, potato and pineapple farmlands of Mana, Jimma, Shebe sombo, Saka, Gojeb and Dedo woredas. The QuEChERS methodology that involves the extraction of the sample with acetonitrile containing 1% acetic acid and anhydrous magnesium sulfate, as well as anhydrous sodium acetate followed by d-SPE cleanup step, were used. Chromatographic separation and quantitative determinations of 4 OPPs and 7 OCPs was performed using gas chromatography-electron capture detector. The validity of the method was evaluated by recovery studies by spiking the target pesticides in tomato, potato and pineapple samples. The obtained recovery values were ranging from 71.22 – 121.56% with the exception of the recoveries of chloroflurenol-methyl and chloropyrifos, which were 65.651% and 67.90%, respectively, in potato matrices. Pesticides including dimethoate (except in Mana and Saka buyo qacama tomato), malathion (except in Saka buyo qacama tomato), p,p'-DDT, endrin, and dieldrin were not detected in all tomato, potato and pineapple samples. Other pesticides such as chloropyrifos, dibutyl chlorendate, p,p'-DDE, chloroflurenol-methyl, endosulfan sulfate and methoxychlor were detected in all samples. But, except dibutyl chlorendate and chloroflurenol-methyl the remaining detected concentrations of other pesticides considered in this study were below the MRL set by EU guidelines. Although the obtained concentrations were safe for most of the analytes, the one way ANOVA result ($p \leq 0.05$) indicated the presence of significant variations among the sampling sites. Generally, the observed result indicates the need for the regular monitoring of pesticide residues since the detected levels indicate as the producers are using these pesticides on their farmlands.

***Keywords*:** *Organochlorine and organophosphorus pesticides; Tomato, potato and pineapple samples; QuEChERS methodology; GC-ECD*

1. INTRODUCTION

1.1. Background of the study

Pesticides are essential for the production of adequate food supply for the increasing world population and for the control of insects, weeds, and rodents, which are harmful in agricultural or horticultural planting [1, 2]. Annually, 2.5 million or more tons of pesticides are used worldwide and of this, more than 1000 different kind of substances are active against pests [3]. Perhaps, still, about 40% of the total potential world food production is lost by pests, plant pathogens, and weeds [4]. Thus, the use of pesticides provides unquestionable benefits for increasing agricultural production [5].

On the other hand, the use of pesticides in excessive amount can pollute the environment and poisons human health. Its presence in food is particularly dangerous due to environmental stability, ability to bioaccumulates and toxicity of pesticides may place human health at greater risk of disease and poisoning [1, 6]. It was reported that about 26 million humans suffer from pesticide poisonings each year [4].

Even if, the developing nations total annual pesticides consumption is only 20% of the world, pesticides poisonings are more serious in these nations. These could be attributed to the inadequate use of occupational safety standards, protective clothing, and washing facilities; insufficient enforcement of safety regulations; poor labeling of pesticides; illiteracy; and insufficient knowledge of pesticide hazards [2, 4]. In Ethiopia, indoor and outdoor applications of pesticides including restricted and banned pesticides is a daily practice to increase productivity and to protect different food items from various pests before and after harvesting [7]. These activities could potentially contaminate different food items produced in the country and in turn may impact public health [7 - 9]. Furthermore, the impact of pesticides in the country are likely to be aggravated by many reasons such as limited knowledge among users on toxicological and chemical properties of these substances; the fact that labels on pesticide containers were in a language which user could not understand or missing; lack of awareness on long-term and indirect effects of pesticides by rural and urban communities as well as on local and national food production systems; and also in order to achieve a better agriculture; some farmers use pesticides incorrectly and excessively or the sale of crops, soon after spraying [2,10].

From those pesticides, organophosphorus (OP) and organochlorine (OC) pesticides are widely used in Ethiopia for control of insect and diseases from different agricultural crops including fruit and vegetables [11]. Since fruits and vegetables are mainly consumed raw or semi-cooked and usually receive a direct application of pesticides in the field or in post-harvest treatment and they may retain a proportion as active pesticide residues in or on their edible portion. It is expected that fruits and vegetables contain higher pesticide residues than other foodstuffs. Pesticide residues can also remain in food after they are applied to crops, even after being washed, stored, processed and prepared, and may result in adverse consequences to the human health [5, 10].

Because of these facts, analysis of residues are mainly carried out for the purpose of enforcement of the food safety regulation and health impact assessment against the maximum residue limits (MRL) [12]. For this purpose different sample preparation procedures have been developed for the analysis of pesticide residues in fruits and vegetables [13]. Among these methods, the QuEChERS methodology was first reported in 2003 [14] and has provided high-quality results for multi-residue analysis of pesticides from fruits and vegetables. The procedure is fast, easy, and an inexpensive [13, 14].

These days, consumers of any nation are becoming increasingly aware of the importance of food safety and are therefore demanding to verify compliance with standards [15]. Thus, in this study, the concentration levels of organochlorine and organophosphorus pesticides residues, in tomato, potato and pineapple of some selected farmlands of Jimma and Kefa zone (particularly, Gojeb), southwestern Ethiopia were investigated for the first time. The target analytes were extracted from the studied samples using QuEChERS and their quantitative determinations were carried out using gas chromatography - electron capture detector.

1.2 Statement of the problem

It is estimated that over 85% (86 million) of Ethiopia's population live in rural areas and depend on agriculture [16] and the sector is the dominant source of foreign currency earnings about 50% to the GDP, about 90% to foreign export, and 80% to employment [17]. But, finding shows that the average crop loss due to pests was estimated to 30 - 40% per year. To face this challenge use of pesticides in the agricultural sector were introduced in Ethiopia in the 1964's [10]. Because of this, different types of pesticides were imported by both private and public companies for agricultural uses [18]. These days, Ethiopia is considered as having the largest accumulations of obsolete pesticides in the horn of Africa. Also in view of the current intensification of agricultural activities and increased intensity of pesticide use, in combination with the abundance of raw fruit and vegetable consumers in the country, the risk posed to humans and the environment from the application of pesticides [19].

Study of levels of pesticide residue in a food item is very limited in Ethiopia. Particularly, the contamination status of fruit and vegetables by pesticide residues in southwest Ethiopia has not yet been reported. Hence the present study hypothesized that application of these pesticides and the historical use of some of the persistent pesticides, such as organochlorines, and organophosphorus resulted in contamination of fruits and vegetables by the pesticides. Therefore, this study was aimed to determine residues of some selected organophosphorus and organochlorine pesticides from tomato, potato and pineapple samples of selected farmlands in Southwestern Ethiopia.

1.3 Objectives

1.3.1 General objectives

- The main objective of the study was to determine pesticide residues in fruit and vegetable samples of southwestern Ethiopia.

1.3.2 Specific objective

The specific objectives of this research were:

- To determine the levels of organochlorine and organophosphorus pesticide residues in pineapple samples of Gojeb, Jimma agricultural research center (JARC), Shebe sombo, Saka and Dedo.
- To determine the levels of organochlorine and organophosphorus pesticide residues in potato samples of Jimma Amenu kebele, Shebe sombo, Saka, Mana, and Dedo
- To determine the levels of organochlorine and organophosphorus pesticide residues in tomato samples of Jimma Amenu kebele, Shebe sombo, Saka, Mana, and Dedo
- To compare the contamination of OCPs and OPPs residues in pineapple, potato and tomato samples with the legally prescribed MRLs.

1.4 Significance of the study

Findings of the study could have the following significances:

- The obtained result could be used as the background information in education and research about the contamination status of pesticide residues in fruits and vegetables in general in food matrices. Means that, the information can be used by other scholars as literature review basis for further research.
- It has its contribution to baseline information for the development of standards regarding maximum organochlorines and organophosphorus pesticide residue limit in fruit and vegetable grown in Ethiopia.
- It contributes to increase the income of Ethiopian farmers, since pesticide residues in horticultural crop products may also have a detrimental effect on the export of agricultural crops.
- To provide some insights on the trend of pesticide use and its impacts on public health and the environment.
- The result can be used by the Ministry of Agriculture and other stakeholders to raise awareness of the need for safe handling and use of pesticides by the farming community. through training and information dissemination for human and environmental safety.
- To the government and other stakeholders in developing appropriate policies to enhance environmental and human safety in pesticide use for sustainable agricultural production.

2. REVIEW LITERATURE

2.1 Fruits and vegetable production in Ethiopia

Various types of fruit and vegetable crops are grown in Ethiopia under rain-fed and/or irrigation systems [19]. Because the country has favorable climate and edaphic conditions for the production of tropical, sub-tropical and temperate vegetables in the lowlands (<1500 meters above sea level), Midlands (1500-2200), and highlands (>2200), respectively [20]. According to Ethiopian Horticulture Development Corporation report commercial production of horticultural crops, including fruits and vegetables, has also been increasing in recent years because of expansion of state farms and increasing private investment in the sector by national and international entrepreneurs [21].

But, it has been noted that smallholders farmers usually use the largest part of their horticultural crops produce for home consumption and sell the surplus. With this fact production of the horticultural crop in the country is integrated into a mixed farming system where different types of crops are produced on the same plot of land or in sequence with other crops in rotation. Depending on availability of land and crop suitability for intercropping, some vegetables are grown either as sole or intercropped with other vegetables or cereals [19]. Even if, the country exported 220,213 tons of vegetables in 2013 and generated 438 million USD the contribution of horticultural crops both to the diet and income of Ethiopians is insignificant [22].

This is because of horticultural crop production in the country has been constrained by a myriad of biotic (i. e., diseases, insect pests, and weeds) and hence, Ethiopian ministry of agriculture recommend the use of agrochemicals. However, some of the used pesticides have a residual effect and may pose a serious threat to the health of the consumers [23]. So to enhance the export capacity and production of fruits and vegetables, the quality (degree of their contamination with hazardous chemicals) of these products is extremely important and is often a factor determining whether or not consumers will buy them. Because, safe food should have above all an appropriate nutritious value and contain the least possible amounts of substances that could be hazardous to health [6, 18, 19].

2.2 Health benefit of fruit and vegetable

Fruits and vegetables possess a protective effect against various degenerative diseases due to the presence of various phytochemicals, carotenoids, vitamins, and minerals [24]. Particularly, the consumption of fresh vegetables gives the consumer a variety of compounds that have a positive influence on human health. For example, phytochemicals found in fresh vegetables and fruit have an anti-inflammatory, enzyme inhibiting and bioactive features capable of combating the activities of oxidants [25].

Due to the presence of several research reports on the biological functions of microelements in the human body in recent years. There has been an increasing interest in the use of these elements as micro-nutrient supplements or functional foods in medical treatment to prevent various diseases such as cancer, cardiovascular diseases, AIDS, Alzheimer's disease, osteoporosis, osteoarthritis, asthma, cataract, and aging [24]. The World Health Organization acknowledges that the global intake of vegetables is less than 20-50% of the recommended amount. In Ethiopia, there is significantly low vegetable intake due to the consumer's preferences for partly because of the rising prices of livestock products such as meat, milk, and eggs, which traditionally forms a major component of most Ethiopians diets and not only the scarcity of vegetables [19, 25].

2.3 Tomato production and its nutritional value

Tomato (***Lycopersicon esculentum*** **Mill.**) belongs to the family ***Solanaceae*** and is the most consumable vegetable crop after potato and sweet potato, occupying the top of the list as canned vegetable having multiple uses. It is one of the most popular vegetables due to its outstanding nutritive value and widely grown in the world [26]. As we know a tomato is not indigenous for Ethiopia rather native to South America but growing in temperate climates worldwide [27]. The introduction of cultivated tomato into Ethiopian agriculture dates back to the period between 1935 and 1940. During that time, 300 tomato varieties were tested in 1969 by Ethiopian Institute of Agricultural Research (EIAR) which was established in 1966. The first record of commercial tomato cultivation is from 1980 with a production area of 80 ha in the upper Awash by Merti Agro-industry for both domestic as well as export markets [28].

In the country the crop is grown between 700 and 2000 m above sea level, with about 700 to over 1400 mm annual rainfall, in different areas and seasons, in different soils, under different weather conditions, but also at different levels of technology (e.g. with furrow, drip or spate irrigation) and yields) [29]. Nowadays, the tomato is widely grown vegetable crop throughout the country and is consumed in every household in different modes, more in certain areas, such as Walo, Hararge, Shawa, Jimma, and Wallaga, which is also an important co-staple food [28].

Tomatoes are known to be a rich source of vitamins, minerals, and carotenoids, especially vitamin C, phosphorus, potassium, and lycopene which is largely responsible for the red color of the fruit [24]. Besides the basic nutritional properties, tomatoes contain bioactive compounds with antioxidant properties such as ascorbic acid, vitamin E, carotenoids (lycopene), flavonoids and phenolic compounds that benefit human health [27]. These compounds may play an important role by inhibiting reactive oxygen species responsible for many important diseases, through free-radical scavenging, metal chelation, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways [30]. But tomato production is under the threat of various insect pests and diseases in the field. To combat insect pests and diseases of this crop and to achieve higher production, many pesticides are used that may leave certain amounts of residues on the crops. These residues, if present in excess, may act as a health hazard to the consumers and may cause chronic diseases [28].

Figure 1 Tomato (*Lycopersicon esculentum Mill*) vegetable (captured during sample preparation)

2.4 Potato production and its nutritional value

Potato (***Solanum tuberosum L***.) which belongs to the family ***Solanaceae*** has been used as a food for more than 10,000 years, starting in South America Peru where it originated. Currently, the top three world leaders in potato production are China (# 1), Russian Federation (# 2) and India (# 3). Potato is increasingly important to world food security in developing countries including Ethiopia, where it supplements or has replaced grain-based diets [31]. It is an important food security, cash income, and a hunger reliever crop in several parts of the country by virtue of its ability to mature earlier than most other crops at time of critical food need; it is high yielding ability in a short season; presence of suitable agro-ecological zones within the country; the availability of labor for its production on large areas of land; and the accessibility of a potential market with considerable added value for its produce [31, 32, 33].

In recent years, the production of this crop is expanding rapidly owing to the presence of improved technologies and expansion of irrigation culture. Potato production in southern Ethiopia is twice a year where, the bulk production is during *Belg* (a short rain season, March–June) season, whereas small production takes place during the *Meher* season (a long rain season, July–October) [31, 32]. Even if, the potato is currently the predominant vegetable in the world in terms of sales, production, and consumption, it is produced mostly for local consumption and local markets in Ethiopia [32].

However, one of the limiting factors of this crop is closely linked to its susceptibility to pests and diseases, which results in the use of large amounts of pesticides throughout its growing cycle [34]. Due to this, in 2009 many research findings explore the possible pesticide residues in potato such as organophosphates, carbamates, and organochlorines [35]. Culturally, the potato is consumed in different forms such as boiled, fried, stewed, salad, etc. and has contributed to satiety, the feeling of fullness that you get from eating and preventing over-eating (obesity). Potato is high in compounds that promote mineral bioavailability such as ascorbate, B- carotene, organic acids, cysteine-rich polypeptides, oxalates, and phytates. In addition to this, it contains diverse antioxidants including vitamin C, polyphenols (anthocyanins and phenolics) [31, 36].

Figure 2 Potato (*Solanum tuberosum L.*) vegetable and plant (captured during sampling and sample preparation)

2.5 Pineapple production and its nutritional value

Pineapple (***Ananas comosus* L.**) belongs to the family ***Bromeliaceae*** is originated in Brazil/Paraguay and it is a perennial crop that can be cultivated any time of the year, so long as soil moisture is available [37, 38]. It is known as the queen of fruits because of its excellent tropical fruit having exceptional juiciness, vibrant tropical flavor, taste and immense health benefits [38, 39]. According to [40], the pineapple plants are drought tolerant and well adapted to the tropical sandy soils with pH ranging from 4.5 to 6.5.

Pineapple is the third most important tropical fruit crop after banana and other citrus fruit, contributing to over 20% of the world production of tropical fruits. In many countries producing the pineapple, nearly 70% is consumed as fresh fruit [38]. The fruits are used mainly for fresh consumption and fruit juice, while in some parts of the world the fermented juice is used to make vinegar and alcoholic spirit; and their juice's composition varies depending on geography, season, process and time of harvest [41]. Various food items like squash, syrup, jelly, Vinegar, alcohol, citric acid, calcium citrate etc. are produced from pineapple. Pineapple is also recommended as a medical diet for certain diseased persons [39].

Because pineapple contains a considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fiber, water, and different minerals that are good for the digestive system and helps in maintaining ideal weight and balanced nutrition [42]. Despite this merite, consuming a large quantity of fresh pineapple juice can cause mouth and esophagus soreness. The irritation results from the combined action of the acids, bromelain enzymes and calcium oxalate crystals. The high level of citric acid in fresh, unsweetened pineapple juice may cause an upset stomach if large quantities are consumed, especially on an empty stomach [41].

However, due to the susceptibility of these cultivars to ***Fusarium guttiform*** (***syn. F. subglutinans f. Sp. Ananas***), which causes ***fusariosis***, is responsible for major losses during cultivation. Due to this pineapple production in large scale by high-input is dependent on the regular and intense use of a number of toxic agrochemicals [39]. That is why this study focuses on this commodity as well as on large-scale pineapple production farmlands particularly Horizone plantation private limited company farm station in Gojeb together with other smallholder farmlands in Southwest Ethiopia.

Figure 3 Pineapple (*Ananas comosus L.*) fruit plant (captured during sampling)

2.6 Pesticides and their classification

Pesticides are agrochemicals widely used in agriculture to protect plant and crops from pests and plant diseases. Pesticides are, by controlling pests, used to increase crop productivity and improve the quality of products [43]. They are different in uses and mechanism of action to the target species. The classification of these agrochemicals is usually based on their intended use such as insecticides (insects), herbicides (weeds), nematocides (nematodes) [44]. Besides their use as pest control, the residue of the applied pesticide could remain in the environment and can pollute the environment and contaminate foods such as fruits, vegetables, water, and soil.

The contamination of food items by hazardous substances such as residues of persistent pesticides is a worldwide public health concern. Some pesticides are hazardous and toxic to human health; exposure to residues of such toxic substances can pose danger to humans and may cause certain diseases [43, 44]. Most pesticides are generally toxic to non-target species including humans. Pesticides affect human health in a number of ways. Some have mild irritant effects in the skin, others affect liver or lung functions and some are carcinogenic. Several pesticides, particularly insecticides, are neurotoxic to insects and humans as well [44]. Exposure to pesticides can occur in a number of ways; residues in food, mixing and loading of pesticides and application of the pesticides, harvesting of pesticide sprayed crops are common ways of exposure to pesticides that could lead to higher risk. Mostly the high exposure by these means occurs in developing countries [43].

2.6.1 Insecticides

Insecticides play a vital role in the control of insect pests. As we compared to other pesticides, insecticides are the most acutely toxic to non-target species including humans. All of the insecticides used today are neuron-toxicants which are acted by poisoning the nervous system of the target organisms [1]. Target sites of toxicity for insecticides to insects are also found in mammals including humans. Because of this insecticides are not species selective towards toxicity. Neurotoxicity is an adverse effect on the central or peripheral nervous system caused by chemical, biological or physical agents [44].

Several studies in developing and developed countries indicate that insecticides, particularly organophosphates, are the most responsible for human poisonings. Because organophosphate and carbamate pesticides are the most widely used insecticides [7, 44].

2.6.1.1 Organophosphate insecticides

The most commonly used pesticides in agriculture are organophosphorus compounds (OPCs). There are more than 100 different organophosphorus pesticides and poisoning of these chemicals can face both intentionally and unintentionally, results in worldwide health problem [45]. They are used to destroy insects such as fleas, lice, flies, and mosquitoes. These chemicals are used for pest control on crops in agriculture and on livestock as well and have high acute toxicity [46].

Target site of OPs insecticides for toxicity is acetylcholinesterase enzyme (AChE), an enzyme whose role is hydrolyzing acetylcholine, a neurotransmitter in the central and peripheral nervous system. The organophosphate insecticides bind with the cholinesterase (ChE) enzyme at the neuromuscular junction and deactivate or inhibit the activity of the enzyme by irreversible phosphorylation. Inhibition of AChE by OPs causes accumulation of acetylcholine at cholinergic synapses, with overstimulation of cholinergic receptors of the muscarinic and nicotinic type [44, 46].

As these receptors are localized in most organs of the body, a cholinergic syndrome ensures, which includes increased sweating and salivation, profound bronchial secretion, bronchoconstriction, miosis, increased gastrointestinal motility, diarrhea, tremors, muscular twitching, and various central nervous system effects. Acute exposure to high doses of OPs may result in long-lasting adverse health effects in the CNS [44].

In addition to the acute cholinergic syndrome, OPs may also cause an intermediate syndrome [46], organophosphate-induced delayed polyneuropathy (OPIDP) whose signs and symptoms include tingling of the hands and feet, followed by sensory loss, progressive muscle weakness and flaccidity of the distal skeletal muscles of the lower and upper extremities, and then ataxia, which may occur 2-3 weeks after a single exposure [44, 46].

Generally, exposure to organophosphate insecticides at low to moderately high doses develop a pesticide-related illness. These mild to moderate symptoms of organophosphate toxicity include nausea, headache, dizziness, blurred vision, abdominal pain, vomiting, and chest tightness, with ChE depression [46].

2.6.1.2 Organochlorine insecticides

Organochlorine pesticides are chlorinated hydrocarbons which are widely used in agriculture and mosquito control. The acute toxicity of OC pesticides is moderate (less than OP pesticides), but chronic exposure causes adverse health effect mainly in the liver [44, 47, 48]. In humans these substances and their metabolite alters the electrophysiological properties and enzymatic neuronal membranes, causing alterations in the kinetics of the flow of Na^+ and K^+ through the membrane of the nerve cell, resulting in the spread of multiple action potentials for each stimulus, causing symptoms such as seizures and acute poisoning death from respiratory arrest [47, 48]. They are highly lipid solubility (lipophilicity), low polarity, low aqueous solubility, chemically stable and persistent in the environment with enduring half-lives [1, 47]. Due to their high environmental persistence and health impact, these compounds have been banned from use in most countries. Nevertheless, continuous monitoring of OCP residues in food is needed because they are persistent in the environment and have a high tendency to accumulate in living organisms and the food chain [11].

2.7 The fate of pesticides in the environment

Pesticides are distributed into four major compartments after applied in the field, water, air, soil, and biota (living organisms) [49]. The amount of fraction of pesticides moved into each compartment depends on the physicochemical properties of the pesticide. Physical processes, such as sedimentation, adsorption, and volatilization plays a vital role in the distribution of pesticides in the environment [49, 50]. Following this, they can be degraded by chemical and biological processes. The physicochemical characteristics of the pesticide (water solubility, its absorptivity to the soil, volatility) and soil characteristics (clay, pH, sand and organic matter) determine the fate of pesticides in the environment. The dissipation of pesticides from the application site creates three major problems: economic loss to farmers, inefficient control of pests, and possible environmental contamination [49].

Generally, solubility, hydrolysis, volatility, photodegradation, microbial degradation, leaching, oxidation/reduction, all these are factors that determine the fate of pesticides in the environment [49 - 52].

In addition to these, environmental weather condition plays a greater role in the fate of pesticides. The specific pesticide has different dissipation rates in tropical and temperate regions. Soils of temperate and tropical regions are investigated for the degradation rate of pesticides under controlled laboratory conditions and show no degradation rate difference [50, 51]. But the field investigation shows higher degradation rate occurs in tropical regions. This shows that pesticide degradation is dependent on environmental weather conditions [51, 52].

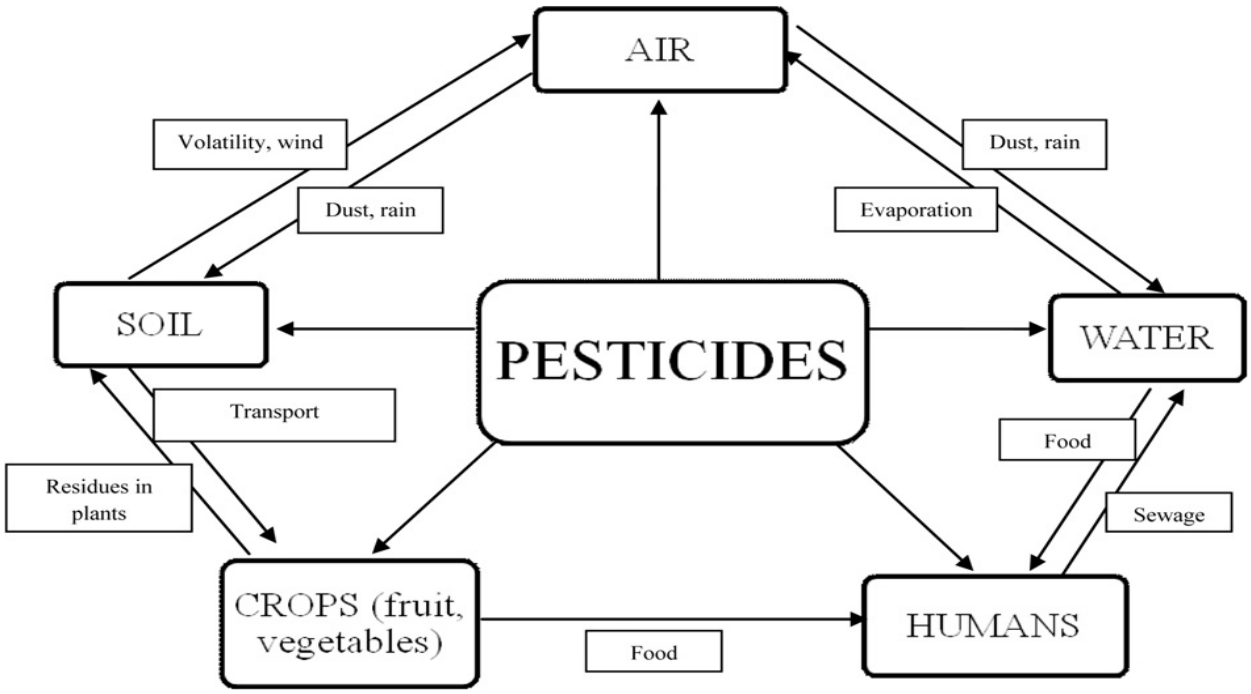


Figure 4 Circulation of pesticides in nature including crops [6]

2.8 Pesticides use and practice in Ethiopian agriculture

The possible source of pesticide exposure could be from occupations as agricultural workers, sprayers, exterminators, formulators, or it could be from living near the farm where pesticides are applied or from consuming pesticide-contaminated food [53]. Various kinds of pesticides are widely used in Ethiopian agriculture. Such as; organophosphates, carbamates and to some extent organochlorides are among the widely used pesticides. Those pesticides that are restricted and banned from use in the developed countries are still used in developing countries [7].

Although, poor storage conditions (e.g. leaking drums, burst open sacks) and stock management are causes for a great risk of contamination and posed a great threat to human health and the environment [1]. Awareness of the farm people on the health impact of pesticides, use, and practice of application and hazards is totally poor. The majority of farmworker believes that the major problem for pesticide application is windy and sunny conditions [7]. This is because of a study on the awareness of farmers towards the health impact of various pesticides in Ethiopia is very limited. However, a few studies show that the health impact of these agrochemicals is not well understood by the users [53].

2.9 Sample preparation in pesticide determination

Sample preparation is the first step in any instrumental analysis, which involves the isolation or extraction of the desired analytes from the sample matrix since they are present at trace concentration (usually μg/kg or less). It helps in the elimination of any interferences and also reduces the volume of extracts, thereby concentrating the analytes [54]. The type, nature, composition of sample and concentration of analytes to be isolated or extracted determines the choice of separation and detection method to be used. This also dictates the type of sample preparation to be employed. Since the efficiency of any analysis is determined by the sample preparation step [55].

On the other hand, in accordance with current trends, the analytical procedures should aim at the miniaturization and simplification of the sample preparation step, while maintaining the high throughput performance, low-cost operation, and improvement of the sample preparation, such as extraction, concentration, isolation of analytes, and clean-up. This effort focuses on sample preparation; where there is a shift from laborious traditional method to new fast and simple approaches, such as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) multi-residue method [54-57].

2.9.1. Principle and application of QuEChERS method

There have been substantial efforts in the past two decades to adapt the existing sample preparation methods and develop new approaches to save time, labor and materials [58]. It has been estimated that the sample preparation step in most determinations consumes approximately 60 – 70 % of the total time required for the analysis [56].

QuEChERS (quick, easy, cheap, effective, rugged, and safe) was first introduced for pesticides residues analysis from fruits and vegetables with high water content and becoming a popular means of sample preparation internationally [59 - 62]. However, more recently it is gaining popularity for the analysis of pesticides and other compounds in a huge variety of food products and other different matrices [14, 63]. QuEChERS method and its modifications are now rapidly developing beyond its original scope of application for multi-residue analysis in various matrices. Both polar and non-polar compounds are extracted simultaneously where initial extraction involves the use of an organic solvent followed by partitioning with the addition of salt mixtures and final clean up.

Nowadays, dispersive solid-phase extraction (d-SPE) is the most widely used method for the clean-up. The d-SPE method is similar to the SPE principle but solid phase such as C_{18}, PSA or GCB is added directly and makes the clean-up process easy. This clean-up process is widely used after the enactment of the QuEChERS extraction method in the multi-residue analysis [59]. The usage of absorbent PSA is standardized along with the addition of magnesium sulfate to remove unwanted substances (sugar, fatty acids, and water) from organic solvents in the GC application. The use of salts such as magnesium sulfate to induce an exothermic mass partition of pesticides from the aqueous to the organic phase is crucial in the procedure [9, 13, 64].

Using this method, a batch of 10−20 samples could be extracted in 30−40 min by a single analyst, hence it is very rapid, the need of using only basic laboratory devices make this sample preparation technique relatively inexpensive in comparison to most traditional extraction methods and low solvent and glassware usage (no chlorinated solvents usage). In contrast to this, since it uses 1 g sample per milliliter of final extract, the concentration of the extract is lower than for the concentrated extracts obtained by use of most traditional procedures [14, 64-66].

2.10 Analytical determination of pesticide

This day, the most important and common methods used for pesticide determination are GC and LC [67]. Liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) is one of the most powerful techniques for pesticide residue analysis in fruits and vegetables. But, this technique is used for highly polar, thermally liable and non-volatile compounds [59]. Despite LC, GC is an analytical technique for separating compounds based on primarily their volatilities [67]. Since its introduction in the late 1960s, GC has an inherent remarkable feature to perform multi-residue analysis [58].

Most sample preparation procedures for GC determination follow the basic steps as outlined here: the food sample is homogenized or blended to obtain a uniform matrix; this will be followed by extraction of the pesticide residue with solvents; a cleanup step is employed to remove interfering matrix components from the GC chromatograms; the elution and/or fractionation of the extracted analytes; concentrate the eluent and re-constitute in a solvent which is compatible with the GC conditions; and finally, the solution containing the pesticide can be introduced into the GC [58, 68].

A number of GC based studies have been reported for pesticide analysis by coupling with various detectors such as electron capture detector (ECD) [5, 9, 12], mass detector (MS or MS/MS) [1, 13, 14, 23], nitrogen phosphorus detector (NPD) and flame photometry detector (FPD). Particularly, ECD is a popular detector due to its sensitivity and specificity for electronegative chlorine atoms [5].

3. MATERIALS AND METHODS

3.1. Descriptions of the study area

The study area for this research work was Jimma zone and one special district of Kefa zone which are among the major fruit producing baskets in southwestern Ethiopia. This study focused on these two zones in some selected potential growing areas of fruit and vegetable. Specifically five districts of Jimma zone; Jimma town (Amenu kebele and JARC), Shebe sombo (Atrogefra kebele), Saka (Buyo qacama Keble), Dedo (Waro kolobo kebele), Mana (Somodo kebele), and one district of Kefa zone Gojeb Horizon plantation private limited company (PLC), which are considered as a source of tomato, potato and pineapple were selected. Map of the study area locating the sampling position is given in Figure 5 below.

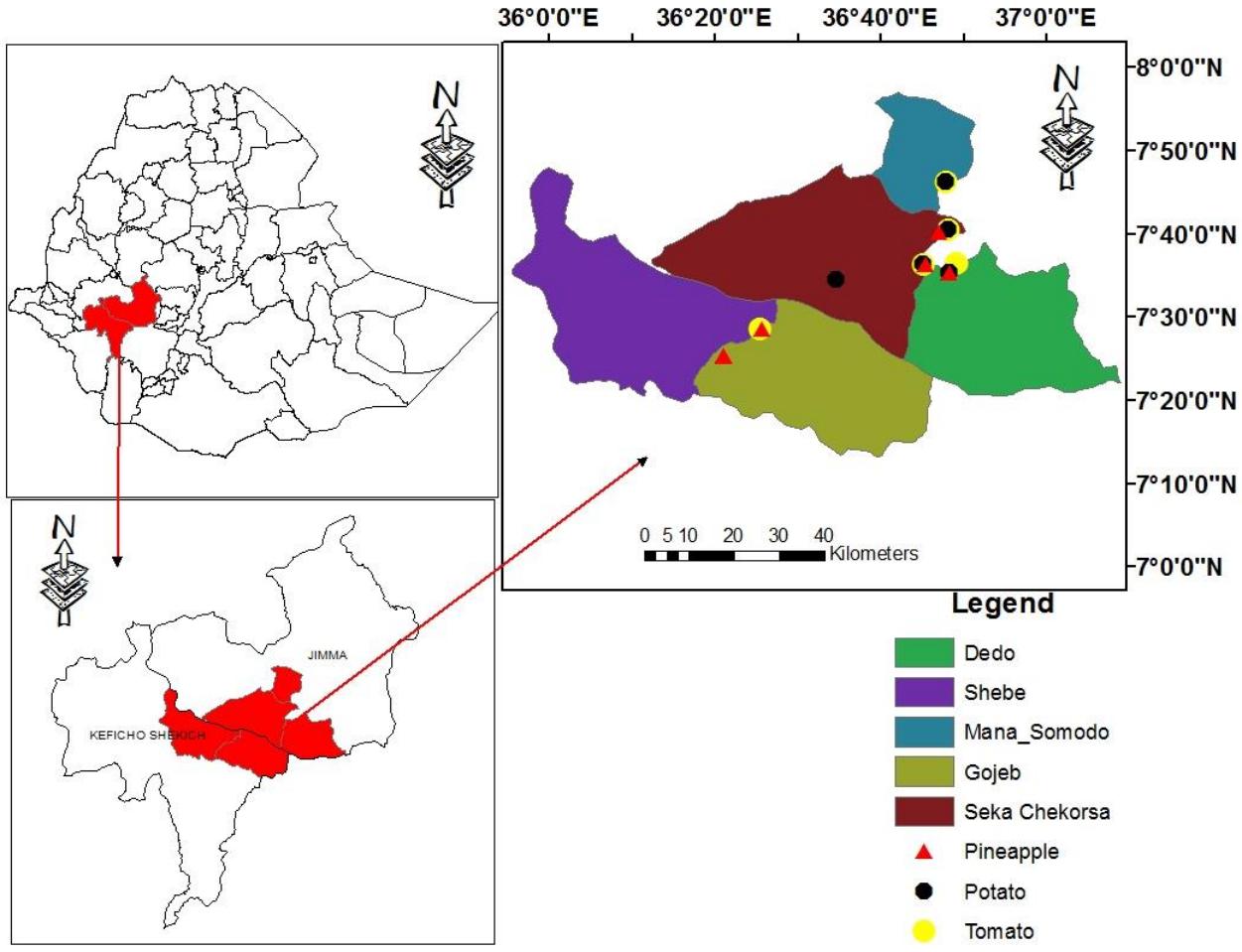


Figure 5 Map of the study area including sample source

3.2 Sampling

Fruit and vegetable samples were collected from the end of April 2018 until mid of May 2018 for residue analysis. Samples were selected purposively from fruit and vegetable farmer's field. Prior to sample collection, through key Informant Interviews conducted with farmers and woredas, agricultural extension personnel's to understand the practice of pesticide use on fruits and vegetables in all districts were organized. Most of the farmers and woreda agricultural extension personnel replayed as they use Malathion, Diazinon, Chloropyrifos, Roundup, Mancozeb, and other OP pesticides. Based on this information representative samples of target fruit and vegetable were randomly collected and then brought to the Jimma university analytical chemistry laboratory. Each representative identical vegetable or fruit sample items were a composite of 3 subsamples of the same commodity collected through random sampling.

3 kg of each tomato, potato, and pineapple per sample was collected and kept below 4 ^{o}C in a refrigerator until the time of sample preparation and subsequent analysis. Blank tomato and potato samples were collected from Dedo, Waro kolobo kebele farmlands and pineapple were from Jimma agriculture research center experimental station plot. All the blank samples were tested for the non-pesticides contaminant before experimental studies. The Codex Alimentarius Commission guidelines (FAO/WHO) was following for sampling and sample preparation [69].

3.3 Chemicals and Reagents

All chemicals and reagents used were high-performance liquid chromatography and analytical grade. Solvents like n-hexane from LOBA Chem (India); acetonitrile from CARLO-ERBA reagents S.A.S; and glacial acetic acid from Blulux laboratories Ltd were purchased. Anhydrous magnesium sulfate and sodium acetate were supplied from BDH Chemicals Ltd (Poole, England). Before use, anhydrous $MgSO_4$ and sodium acetate were baked for 5 h at 500 ^{o}C in a muffle furnace to remove possible phthalate impurities.

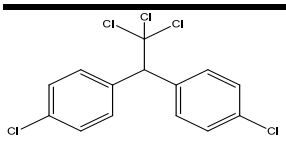
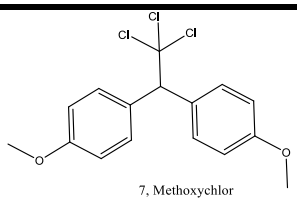
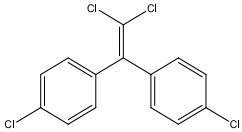
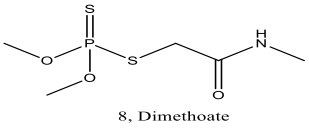
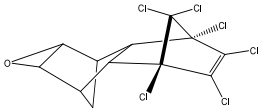
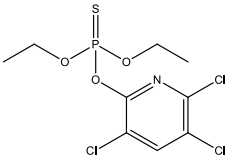
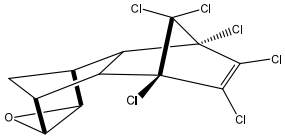
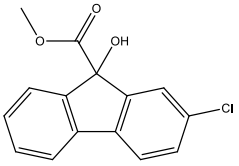
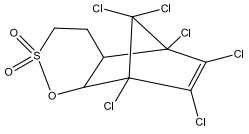
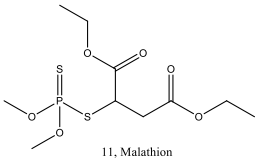
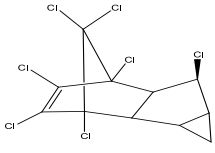
15 mL dispersive solid phase extraction (d-SPE) tubes packed with a mixture of 400 mg primary and secondary amines (PSAs), 1200 mg $MgSO_4$, 400 mg octadecyl (C_{18}) and 45 mg graphitized carbon black (GCB) were purchased from Agilent technologies, US (USA).

High purity pesticide reference standards such as DDT, DDE, endosulfan sulfate, endrin, dieldrin, methoxychlor, dibutyl chlorendate, dimethoate, malathion, chloropyrifos, and chloroflurenol-methyl were supplied from Sigma Aldrich (St. Louis, MO USA). Table 1 shows the structure, melting point, boiling point and molar mass of pesticides under study.

3.4. Solution preparation

Individual Stock standard solutions containing 1000 mg/L of each organochlorine and organophosphorus pesticides were separately prepared by dissolving accurately weighed 10 mg of each pesticide in 10 mL volumetric flask with methanol (except dimethoate, chloropyrifos, malathion and chloroflurenol-methyl which were dissolved in acetonitrile) and then stored in a refrigerator below 4 ^{0}C. Of these stock solution, an intermediate standard solution containing a mixture of 5, 10, 20, 30 and 40 mg/L was prepared by diluting an appropriate volume of each standard in acetonitrile and then, the solution was stored in the refrigerator at 4 ^{0}C. The rest of the pesticide working solutions were prepared by dilution of the intermediate standard solution to appropriate volumes. 1% acetic acid (HOAc) in acetonitrile was also prepared by mixing 10 mL HAOc and 990 mL of acetonitrile.

Table 1 Structure, melting point, boiling point and molar mass of the pesticides under study

Compound name and structure	MM (g/mol)	MP ^{o}C	BP ^{o}C	Compound name and structure	MM (g/mol)	MP ^{o}C	BP ^{o}C
 1, p,p' DDT	354.48	108.50	260	 7, Methoxychlor	345.65	87	decom
 2, p,p' DDE	318.03	194	601.7	 8, Dimethoate	229.26	45	117
 3, Dieldrin	380.93	177	385	 9, Chloropyrifos	350.58	43	160
 4, Endrin	380.91	200	deco at 245	 10, Chlorflurenol-methyl	274.04	152	NA
 5, Endosulfan sulphate	422.90	182		 11, Malathion	330.36	29	157
 6, Dibutylchlordate	387.33	-95	40				

Where: MM = molar mass: MP = melting point: BP = boiling point: decom= decomposes: NA= not available

3.5. Instruments and equipment

The following instruments and equipment were used while conducting this work such as GC-ECD: model 7890A Agilent Technologies (China); centrifuge(s)(capable of holding 50 mL and 15 mL centrifuge tubes used for extraction: model KARL KOLB D- 6072 (Germany and PLC-02,Taiwan, respectively); KERN and SOHNGMBH D - 72336 sensitive balance (capable of accurately measuring weights from 10 to 220 mg within ± 0.01 mg), Balingen; freezer (capable of continuous operation < 4 °C); muffle furnace (capable of 500 °C operation): model KARL KOLB D- 6072; oven used for drying materials; Rotary evaporator: model LABOROTA 4000 (Buchi, Switzerland); Elma sonicator: model D-78224 (Germany); GEEP juice juicer: model (China); Vortex mixer obtained from Fisher scientific: model FB15024 (Belgium) and Agilent Technology 2 mL amber cup vial. Other equipment such as falcon plastic centrifuge tube (15 and 50 mL) volume and different size micropipette were used for sample preparation.

3.6. Gas chromatography operating conditions

All pesticide residue analyses were performed using gas-liquid chromatography with an electron capture detector having an ALS auto-sampler. An HP-5 capillary column (30 m × 0.25 mm inner diameter; 0.25-mm film thickness) coated with 5% phenyl methyl siloxane (model 19091J-433; Agilent) was used in combination with the following oven temperature program: initial temperature of 80 ^{o}C, ramped at 30 ^{o}C min^{-1} to 180 ^{o}C, ramped at 3 ^{o}C min^{-1} to 205 ^{o}C, held for 4 min, ramped at 20 ^{o}C min^{-1} to 290 ^{o}C, held for 8 min, ramped at 50 ^{o}C min^{-1} to 325 ^{o}C. The total GC run time was 27.92 min. Helium (99.99% purity) was used as a carrier gas at a flow rate of 20 mL min^{-1} and nitrogen as a makeup gas at a flow rate of 60 mL/min. An aliquot of 1 µL was injected in splitless mode at a split less ratio of 50:1 and injection temperature of 280 ^{o}C. The pesticide residues were detected with µ-ECD operating at a temperature of 300 ^{o}C.

3.7. Sample extraction and clean-up procedure

Tomato, potato, and pineapple samples were roughly cut into four equal segments (quartered) with stainless steel knife on cutting board before blending to facilitate the subsequent processing. Opposite segments were discarded in order to reduce the bulk of the material needing to be processed. Then the remaining portions were mixed using a juicer machine before weighing 10 g for the extraction procedure. The juicer was washed prior to the next use, to avoid cross-contamination. Only the edible portions were included for the analysis and even the bruised or rotten parts were removed [69].

Extraction and partitioning were carried out according to a modified version of the QuEChERS procedure with the d-SPE clean-up method as per the official method of AOAC 2007.1 [70]. A 10 g portion of the chopped and homogenized sample of tomato, potato, and pineapple was weighed in a 50 mL falcon polypropylene conical centrifuge tube on a sensitive analytical balance. Next, 10 mL of MeCN containing 1% glacial acetic acid (v/v) in each sample was added using a micropipette and samples were shaken by hand for 1 min to increase contact between the solvent and the sample. Afterward, the pre-weighed mass of the following was then added: 6 g of $MgSO_4$ and 1.5 g of anhydrous NaOAc [70].

The sealed tubes were shaken vigorously for about 1 min using vortex mixer to increase sample throughput and centrifuged for 10 min at 4000 rpm and 6 mL of the supernatant was transferred to a 15 mL d-SPE centrifuge tube containing 400 mg primary and secondary amines (PSAs) sorbents, 1200 mg $MgSO_4$ 400 mg octadecyl (C_{18}) and 45 mg graphitized carbon black (GCB). The 15 mL d-SPE cleanup tube was shaken in a vortex for 1min and the system was centrifuged for 10 minutes at the same rpm. The clean-up was done to remove extraneous materials from the extract before analysis. A 3 mL aliquot of the cleaned extract was then taken using round bottom flask and evaporated to dryness using a rotary evaporator at a temperature of 35 ^{o}C.

Then cleaned extract was reconstituted with 1.5 mL volume n-hexane for solvent exchange and an aliquot of 1 mL volume of clean extract was transferred to an amber vial [70]. Finally, the extract was then put into an auto-sampler vial for GC analysis.

One micro-litter of the clean extract was injected for the pesticide residue analysis on gas chromatography and with this treatment, the sample equivalent (mg/g) extract was calculated based on the formula suggested by Schenck and Howard-King [9].

$$\mathbf{Y = a/b\ x/z}$$

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

3.8 Validation of Method

Validation is the process of verifying that a method is fit for the intended purpose. The biggest advantage of method validation is that it builds a degree of confidence, not only for the developer but also to the user [71]; here are typical parameters studied for method validation; linearity, range, precision (repeatability), accuracy (Recovery) and sensibility (limits of detection and quantification). All the validation work analyses were carried out using the pesticide-free tomato, potato and pineapple samples.

Linearity was studied by constructing analytical curves using matrix matched calibration by spiking appropriate volume of pesticide standard mixture on a sample of pineapple matrix extract in six concentration levels. An external calibration, in the same concentrations, was also performed by the dilution of the standard solution of pesticides in hexane for comparison purposes. Recalibration curves were run within a different batch of samples to check that the correlation coefficient was kept above $r^2 = 0.99$ or better. All the Spiked and blank samples were extracted in duplicate (experimental replicates) and each extract was then injected in duplicate (instrumental replicates) and the mean concentration was computed accordingly at each of the six concentration levels.

Sensitivity was obtained from the slope of the calibration curve at the concentration of interest. Detection limits of the method were also assessed based on the lowest concentrations of the residues in each of the matrices that could be reproducibly measured at the operating conditions of the GC. Determination of the S/N ratio was performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected [71].

Accuracy data were obtained with recovery tests performed by spiking appropriate volume of a mixture of pesticide standards in hexane and pesticide free tomato, potato and pineapple sample of extract at the concentration levels of 75 ng mL^{-1}. By following these, samples were subjected to the whole procedure (extraction procedure and d-SPE clean-up). The method repeatability was evaluated through the relative standard deviation (RSD %) associated with pesticide measurements conducted during the recovery test. The precision in the context of the present study can be regarded as repeatability of the method. Repeatability is defined as the closeness of agreement of independent test results under the same method on replicated analytical portions in the same laboratory by the same operator using the same equipment within short intervals of time [12].

In all batches of OCs and OPs pesticides residues analysis, reagent blanks, procedural matrix blanks and duplicate samples with duplicate injection were included. For the reagent blanks (Figure 11) in each extraction procedure, no OC / OP pesticides were detected, meaning that there were no interfering species in the reagents. All extracts were kept frozen until quantification was achieved.

3.9 Statistical analysis

All the detected entire data were statistically evaluated by Statistical Analyses Software SAS (Version 9.0) by applying a coefficient of variation (CV), F test and least significant difference test at $p < 0.05$ with $\alpha = 0.05$ (other SAS parameters mentioned in Appendix part III). The design was completely randomized (RCBD), having tomato, potato, and pineapple together with sample location, as treatments and replication is taken as a duplicate experiment with duplicate instrumental injection ($n = 4$).

4. RESULTS AND DISCUSSION

4.1 Validation study

The peaks were identified by comparison of the retention times with those in the corresponding standards: typical chromatogram of the mixture of pesticide standards with their corresponding retention time in a minute are shown in Figure 6. Chromatographic responses to certain pesticides have been shown to be influenced by the effects of matrices, which are themselves simultaneously influenced by other factors such as pesticide characteristics, matrix type, matrix/analyte concentration, variation in sensitivity of ECD detector during a run and probably caused by contamination with matrix components [72]. In this study the use of matrix-matched calibration standards was done to compensate for the matrix effect, i.e., signal suppression or enhancement of studied pesticides in matrix solution [73].

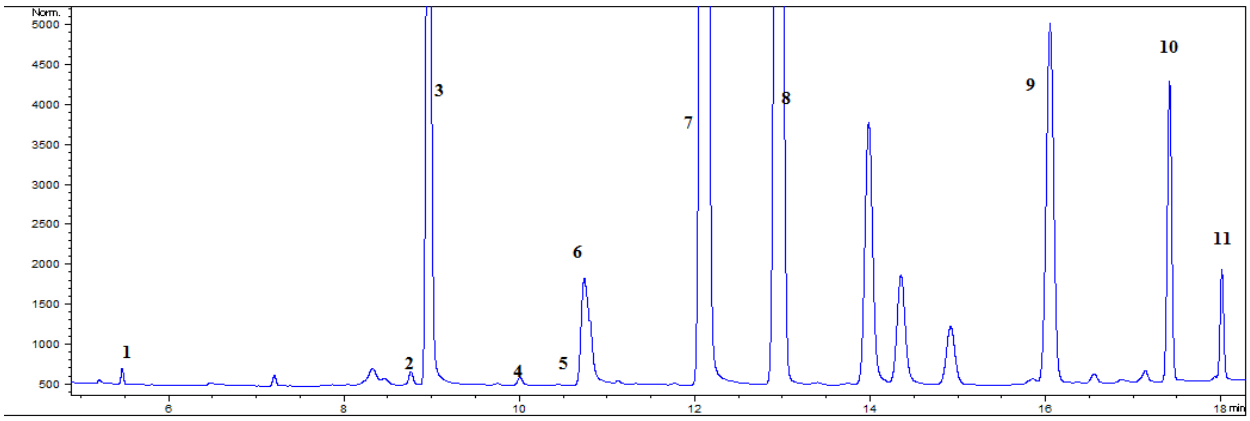


Figure 6 Chromatogram description of target analytes with their retention time (t_R), in n- hexane were: (1) Dimethoate (5.480); (2) Malathion (8.771); (3) Chloropyrifos (8.974); (4) Dibutyl chlorendate (10.015); (5) 4,4-DDE (10.446); (6) chlorflurenol-methyl (10.749); (7) p,p-DDT (12.102); (8) Endrin (12.951); (9) Endosulfan sulfate (16.046); (10) Dieldrin (17.409) and (11) Methoxychlor (18.005)

4.1.1 Linearity study

Calibration curves have been produced for quantification. Linearity has been observed all along the area of concentration studied depending on the target pesticide chemicals. These ranges of concentrations were selected in function of the sensitivity of the gas chromatography towards each pesticide from the correlation coefficient (r^2) of the linear regression. Table 2 shows the analytical performances of the method.

Table 2 Analytical performance characteristics of the utilized method Unit for linearity, LOD, and LOQ are ng/g.

Pesticide list	LDR	r^2	LODs	LOQs
Dimethoate	8.00 – 1600	0.998	0.90	3.00
Malathion	6.00 – 1200	0.999	1.65	5.51
Chloropyrifos	4.00 – 800	0.997	0.03	0.10
DBC	8.00 – 1600	0.999	0.91	3.04
p,p'-DDE	1.00 – 200	0.996	1.21	0.03
Chloroflurenol -methyl	8.00 – 1600	0.999	0.01	0.04
p,p'-DDT	2.00 – 400	0.997	0.01	0.03
Endrin	2.00 – 400	0.997	0.01	0.04
Endosulfan sulfate	2.00 – 400	0.998	0.02	0.06
Dieldrin	4.00 – 800	0.999	0.12	0.39
Methoxychlor	2.00 – 400	0.997	0.09	0.30

As described in the previous section, calibration was performed by the use of matrix-matched standards which prepared at the concentrations of six points. All the residue concentrations in this study were calculated using the matrix-matched standards calibration curve generated from the peak area versus the working solution concentrations. Because the most common method used to prevent matrix effects involves the use of matrix-matched calibration standards [73]. Calibration curves of the studied analytes (shown in appendix part II**)** show satisfactory linearity over selected concentration range with regression correlation coefficients (r^2) ranging from 0.996, for p,p'- DDE, to 0.999 obtained for malathion, DBC, chloroflurenol-methyl, and dieldrin (Table 2).

4.1.2 Limit of detection and quantification study

According to [71] limit of detection is the lowest concentration of analyte that can be detected confidently and also limit of quantification is lowest concentration level at which the measurement is quantitatively detected. In this study, LOD and LOQ of the method were calculated as three times and ten times the signal to noise ratio value, respectively. As indicated in Table 2, the limit of detection of OPPs were ranged from 0.01 to 1.65 ng/g and OCPs were ranged from 0.01 to 1.21 ng/g, is somehow close to MRLs value set by the EU, indicating that the method is suitable for quantification of both selected OPPs and OCPs in fruit and vegetable samples. The limit of quantification values of OPPs and OCPs were ranged from 0.04 - 5.51 ng/g and 0.03 - 4.03 ng/g, respectively. Most of the LOD and LOQ values which are calculated from S/N ratio were below the lowest standard concentration, which indicates that the analytical method is able to detect and quantify still lower concentrations from the food matrices [9].

4.1.3 Recovery study

Table 3 The percent recoveries and precision of the analytical methods considered

Pesticides	Spiking level (ng g^{-1})	Recovery (%) in Tomato	Recovery (%) in Potato	Recovery (%) in Pineapple
Dimethoate	8	114.83 (4.95)	73.22 (2.22)	78.78 (1.77)
Malathion	6	82.13 (2.78)	91.11 (6.80)	93.16 (4.15)
Chloropyrifos	4	82.22 (0.61)	67.90 (13.56)	98.10 (7.45)
DBC	8	76.38 (4.56)	85.21 (4.22)	84.72 (1.60)
p,p'-DDE	1	96.85 (2.94)	72.19 (9.49)	73.51 (9.59)
Chloreflurenol-methyl	8	68.54 (1.75)	65.65 (5.00)	121.56 (8.35)
p,p'-DDT	2	81.92 (2.95)	81.51 (8.42)	85.36 (10.87)
Endrin	2	84.95 (5.69)	99.78 (9.27)	79.82 (6.61)
Endosulfan sulfate	2	76.99 (6.81)	82.82 (6.21)	76.02 (1.69)
Dieldrin	4	107.05 (3.93)	71.22 (15.35)	96.99 (5.12)
Methoxychlor	2	78.04 (7.38)	90.39 (5.38)	91.88 (11.09)

Values in parentheses are relative standard deviation (% RSD).

The concentration of the spiking levels, recovery data and RSDs values obtained are shown in Table 3. Recoveries (n = 4) were calculated as follows: % Recovery = $[(C_f - C_u)/C_s]*100$, where C_f = concentration of pesticides measured in the fortified sample, C_u = concentration of pesticides measured in the unfortified sample (set to zero), and C_s = concentration of pesticides added to the fortified sample [74]. Regarding the acceptance criteria set in SANCO's procedure [75], the average recovery should be within the range of 70 to 120% and RSD < 20%. Recoveries of malathion, chloropyrifos, dimethoate, p,p'-DDT, p,p'- DDE, endrin, dieldrin, endosulfan sulfate, dibutylchlorendate, and methoxychlor were ranged from 76.38% to 114.83% except for Chloroflurenol - methyl (68.54%) with RSD values below 7% in tomato sample.

Similarly, in potato sample matrices, recoveries of all eleven target pesticides under study were ranged from 71.22% to 99.78% except Chloroflurenol - methyl (65.65%) and chloropyrifos (67.90%) with RSD values below 15%. This low recovery of chloroflurenol-methyl and chloropyrifos may be because of losses during the extraction and clean-up steps with C_{18} and PSA or maybe because of matrix effect [9, 12]. However, according to [12] report, for the pesticides that had less than 100% recovery percentage, the difference could be attributed to losses of the analyte during various stages of the analysis. Despite the recovery of other two matrices (tomato and potato), recovery of pineapple gave good results ranging 73.51% to 121.56% with RSD values below 11%, which suggests that the extraction procedure could be appropriate for use in the routine analysis of the targeted pesticide residues in fruit and vegetable samples. Therefore, using 1% acetated acetonitrile (modified QuEChERS method) as an extracting solvent gave good results, which as observed from the recovery studies.

4.2 Analysis of real fruit and vegetable samples

The occurrence of selected organochlorine and organophosphate pesticides was studied in the selected farmlands of Jimma and Kefa zones in southwest Ethiopia. Organophosphate compounds have the advantage of being more rapidly degraded in the environment than organo-chlorine compounds. Organochlorine pesticides, which over a decade ago were being used in Ethiopia, are highly persistent. Most of them have been banned, yet their residues still appear as pollutants in food as well as in the environment [9]. Residue levels of these compounds in fruits and vegetables are listed in Tables 4, 5 and 6. We determine a total of 15 samples consisting of different commodities that were analyzed for seven organochlorine and four organophosphate pesticide compounds.

Table 4 Mean concentration level in (ng/g ± SD) of OPPs and OCPs from tomato samples

Analytes	Sampling sites							
	JA	MN	SS	SQ	DD	LSD	EU	CAC
Dimethoate	ND	58.74 ± 3.38	ND	6.37 ± 2.25	ND	-	10.00	500.00
Malathion	ND	ND	ND	18.11 ± 2.75	ND	-	20.00	500.00
Chloropyrifos	2.97 ± 0.03^{a}	2.76 ± 0.58^{ab}	2.96 ± 0.03^{a}	2.27 ± 0.02^{c}	2.49 ± 0.02^{c}	0.41	10.00	NA
Dibutyl chlorendate	2372.22 ± 3.55^{d}	2623.74 ± 7.61^{b}	2587.09 ± 2.64^{c}	2647.14 ± 3.83^{a}	2238.72 ± 2.93^{e}	7.12	NA	NA
p,p'-DDE	1.33 ± 0.01^{b}	1.26 ± 0.21^{b}	1.31 ± 0.07^{b}	1.20 ± 0.02^{b}	1.64 ± 0.17^{a}	0.18	50.00	NA
Chloroflurenol-methyl	430.33 ± 2.98^{d}	1585.63 ± 6.20^{a}	423.65 ± 1.50^{d}	456.33 ± 2.90^{c}	785.57 ± 8.52^{b}	7.62	NA	NA
p,p'-DDT	ND	ND	ND	ND	ND	-	50.00	NA
Endrin	ND	ND	ND	ND	ND	-	10.00	50.00
Endosulfan sulfate	0.94 ± 0.03^{c}	1.55 ± 0.21^{a}	1.46 ± 0.07^{ab}	1.28 ± 0.02^{b}	1.53 ± 0.15^{a}	0.20	50.00	500.00
Dieldrin	ND	ND	ND	ND	ND	-	10.00	100.00
Methoxychlor	2.88 ± 0.18^{bc}	3.25 ± 0.04^{a}	3.01 ± 0.14^{b}	2.84 ± 0.08^{c}	1.71 ± 0.03^{d}	0.14	10.00	NA

Where; MN = Mana: JA = Jimma Amenu: SS = Shebe sombo: SQ = Saka buyo qacama: DD = Dedo: LSD = Least Significant Difference: ND = Not Detected: NA =Not Available: CAC = Codex Alimentarius Commission (in ng/g) [76]: EU = European Union (in ng/g) [77]: MRL = Maximum Residue Limit: SD = Standard Deviation

Table 5 Mean concentration level in (ng/g ± SD) of OPPs and OCPs from potato samples

Analytes	Sampling sites							
	JA	MN	SS	SQ	DD	LSD	EU	CAC
Dimethoate	ND	ND	ND	ND	ND	-	10.00	50.00
Malathion	ND	ND	ND	ND	ND	-	20.00	500.00
Chloropyrifos	2.07 ± 0.11^{ab}	2.15 ± 0.02^{a}	1.73 ± 0.03^{c}	1.91 ± 0.05^{bc}	1.89 ± 0.32^{bc}	0.22	10.00	2000.00
Dibutyl chlorendate	2858.92 ± 5.26^{b}	2881.13 ± 3.89^{a}	2262.00 ± 6.87^{d}	2328.79 ± 11.40^{c}	1866.15 ± 12.27^{e}	12.29	NA	NA
p,p'-DDE	1.36 ± 0.07^{b}	1.38 ± 0.04^{b}	1.68 ± 0.11^{a}	1.41 ± 0.22^{b}	1.13 ± 0.15^{c}	0.15	50.00	NA
Chloroflurenol-methyl	799.99 ± 1.20^{b}	841.04 ± 2.05^{a}	772.89 ± 1.18^{c}	767.58 ± 6.20^{d}	633.19 ± 0.74^{e}	4.58	NA	NA
p,p'-DDT	ND	ND	ND	ND	ND	-	50.00	NA
Endrin	ND	ND	ND	ND	ND	-	10.00	50.00
Endosulfan sulfate	1.44 ± 0.03^{b}	1.43 ± 0.06^{b}	1.61 ± 0.08^{a}	1.51 ± 0.11^{ab}	1.43 ± 0.04^{b}	0.11	50.00	50.00
Dieldrin	ND	ND	ND	ND	ND	-	10.00	100.00
Methoxychlor	2.72 ± 0.24^{a}	2.29 ± 0.09^{b}	2.52 ± 0.31^{ab}	2.49 ± 0.12^{ab}	2.68 ± 0.24^{a}	0.37	10.00	NA

NOTE: Means with the same letter are not significantly different

Table 6 Mean concentration level in (ng/g ± SD) of OPPs and OCPs from pineapple samples

Analytes	Sampling sites							
	JARC	GH	SS	SQ	DD	LSD	EU	CAC
Dimethoate	ND	ND	ND	ND	ND	-	10.00	5000.00
Malathion	ND	ND	ND	ND	ND	-	20.00	7000.00
Chloropyrifos	2.95 ± 0.05^{a}	2.12 ± 0.10^{e}	2.26 ± 0.03^{d}	2.61 ± 0.02^{c}	2.78 ± 0.03^{b}	0.07	10.00	1000.00
Dibutylchlorendate	2672.53 ± 6.23^{b}	2749.67 ± 3.80^{a}	2504.62 ± 7.35^{d}	2580.88 ± 9.53^{c}	1542.46 ± 9.53^{e}	10.28	NA	NA
p,p'-DDE	1.89 ± 0.01^{a}	1.93 ± 0.01^{a}	1.41 ± 0.02^{d}	1.77 ± 0.02^{b}	1.56 ± 0.06^{c}	0.05	50.00	NA
Chloroflurenol-methyl	664.19 ± 6.63^{c}	1355.57 ± 6.20^{a}	738.15 ± 2.63^{b}	614.18 ± 7.05^{d}	367.88 ± 7.05^{e}	10.02	NA	NA
p,p'-DDT	ND	ND	ND	ND	ND	-	50.00	NA
Endrin	ND	ND	ND	ND	ND	-	10.00	50.00
Endosulfan sulfate	1.47 ± 0.01^{d}	1.27 ± 0.01^{e}	2.21 ± 0.01^{a}	1.90 ± 0.13^{b}	1.75 ± 0.14^{c}	0.13	50.00	5000.00
Dieldrin	ND	ND	ND	ND	ND	-	10.00	50.00
Methoxychlor	8.78 ± 0.30^{a}	8.72 ± 0.73^{a}	2.83 ± 0.18^{d}	7.75 ± 0.05^{b}	5.50 ± 0.01^{c}	0.50	10.00	NA

NOTE: Means with the same letter are not significantly different*; Where; GH = Gojeb Horizion plantation PLC*

The results for pesticide residues that were detected in the three types of fruit and vegetable crops are shown in Table 4, 5 and 6. Residues mean concentration of chloropyrifos in tomato samples of Jimma Amenu, Mana and Shebe sombo woreda was significantly different from Saka buyo qacama and Dedo woreda. The p,p'- DDE contamination status of Dedo tomato sample was significantly higher than the other sites of the sample as methoxychlor in Mana. There was no significant difference of endosulfan sulfate residue level in Mana, Shebe sombo and Dedo. Relatively high residue concentrations of dibutyl chlorendate and chloroflurenol-methyl were found in all the samples. Both dimethoate (except Saka buyo qacama and Mana) and malathion (except Saka buyo qacama) were not detected in all the site sample. In this study p,p'-DDT, endrin, and dieldrin were not detected, because, it was below the detection limit of the instrument.

Similarly, The p,p'- DDE contamination status of Shebe sombo potato sample was significantly higher than the other sites of the sample. The mean concentration of chloropyrifos in potato samples of Jimma Amenu, Saka buyo qacama, and Dedo woreda indicated that there was no significant difference between them statistically. The level of endosulfan sulfate and methoxychlor concentration in the woredas was ranged from 1.43-1.61 and 2.29-2.72 ng/g, respectively. The dibutyl chlorendate and chloroflurenol-methyl potato sample residue concentration are relatively high as tomato sample (2881.13 and 841.04 ng/g, respectively) in the sample collected from Mana than the other Woreda samples. As indicated in Table 5 above, the mean values with the same row followed by the same letters are not significantly different at ($p < 0.05$). In all the sampling site of potato samples dimethoate, malathion, p,p'-DDT, endrin, and dieldrin were not detected.

The contamination distributions of chloropyrifos concentration in pineapple obtained from Woreda's sample ranged from 2.12-2.95 ng/g in Gojb Horizion plantation and Jimma agricultural research center, respectively. The pineapple samples were contaminated with the dibutyl chlorendate up to 2749.67 ng/g in GH, the insecticide chloroflurenol-methyl up to 1355.57 ng/g in GH, metabolite of DDT insecticide DDE up to 1.93 and 1.89 ng/g in GH and JARC respectively, the organochlorine endosulfan sulfate up to 2.21 ng/g in SS and the methoxychlor up to 8.78 ng/g in JARC sample.

The result of JARC methoxychlor means residue was not significantly different from GH sample. dimethoate, malathion, p,p'-DDT, endrin, and dieldrin was the pesticide that did not show up in detectable amounts in this commodity crop.

In the case of organophosphorus pesticides found in all crop samples, chloroflurenol-methyl was the most predominant pesticide residues found in the analyzed samples with a maximum concentration of 1585.63 ng/g MN tomato and a minimum value of 367.88 ng/g in DD pineapple sample. Next, to chloroflurenol-methyl is dimethoate in MN tomato, malathion in SQ tomato and chlorpyrifos. chloropyrifos is a broad spectrum systemic insecticide widely used to control agricultural pests in different crops. In general, it is used worldwide to control a wide range of pests, such as cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants, and lice [78, 79]. The mean residue level of chloropyrifos ranges between 1.73 (potato SS) to 2.97 ng/g in the JA tomato samples.

No residue of dimethoate and malathion was detected in any of crop field tomato, potato and pineapple samples studied except tomato sample of MN and SQ woreda dimethoate residue of 58.74 ± 3.38 ng/g and 6.37 ± 2.25 respectively and SQ woreda Malathion residue of (18.11 ± 2.75 ng g^{-1}). Moreover, tomato, potato and pineapple samples collected from different sites of the farmland contained different concentrations of OPPs. Specifically, a tomato sample which was collected from, MN contains the highest concentrations of the studied pesticides (Chloreflurenol - methyl) than other sampling sites and crops.

Compared with recent studies in some other countries (Bangladesh [26], Ghana [80], Egypt [81] and Bolivia [82]; Table 7), all fruit and vegetable items in the sample site under study contained lower concentrations of all OPs pesticide residues. This might be because of less contamination of the food items by the OPPs. Despite, the other OPPs studied Chloroflurenol - methyl has been exceeded from EU default MRLs in all the samples. This could be attributed to the presence of a source of theses OPPs into the crop. Regardless of the overuse of the pesticides and the persistence of these pesticides which indicated that not all farmers follow the legal practices. dimethoate and malathion concentration in all tomato, potato, and pineapple are below EU guidelines except MN tomato sample which was below CAC MRLs guidelines.

However, in none of the samples, the MRLs allowed for Chloropyrifos, have been exceeded and thus, according to the EU legislation the levels of these OPPs residues cannot be considered a serious public health problem.

Some of the persistent OC insecticides, like endosulfan sulfate, DDE and methoxychlor were detected in all tomato, potato and pineapple samples but at very low levels, very close to the LOQs (Table 4, 5 and 6). However, we had feared to detect organochlorines such as DDT, endrin, and dieldrin in all 3 crops due to poor handling of pesticides. Fortunately, we did not find pesticides from this group or any other obsolete pesticides as was the case in other Ethiopian studies on other crops done by [9]. Thus, no possible comparison of their content in any of the tomato, potato and pineapple samples were done.

But, even if the residue level of p,p'-DDT was not detected in all of the sample and sampling sites, the sum of the total DDT residue level is contributed from a different mean concentration level of its metabolite p,p'-DDE. Although DDT is officially banned for agricultural application in Ethiopia, contamination of food still occurs. This contamination might be explained by indoor spraying of DDT for malaria prevention and by illegal use from obsolete pesticide stocks [9]. This low occurrence of the metabolite (p'p-DDE) compared to the parent compound (p'p-DDT) revealed that the historical use of DDT in the study area [43].

This means that the presence of these persistent compounds by this amount, mainly used in the past, is not critical nowadays and only a few traces are still detected in the samples. Therefore, we can say that no samples contained concentrations of pesticides which alone or together would lead to exposures that exceeded the acceptable daily intake or the acute reference dose except dibutyl chlorendate.

Specifically, among the various organochlorine pesticides in the present study, dibutyl chlorendate is the predominant compound in all the samples at all locations. The detected levels of it varied greatly. For instance, the minimum value for it was detected in pineapple sample collected from Dedo woreda, 1542.46 ng/g and the maximum of 2881.13 ng/g was found in Mana woreda potato sample. But in the present study, out of a total of ten farmers interviewed, none of them were using such organo-chlorine pesticides. This might be explained by higher residues could result from the historical use and contamination, particularly by those compounds demonstrating environmental persistence and by the accumulation of obsolete pesticides [83].

In this study used MRLs stated by EU is more stringent and specific to the individual commodity of fruit and vegetable than CAC guidelines which are stated, general. MRLs are permissible values to evaluate the safety of pesticide residues in fruits and vegetables [84]. However, for most of fruits and vegetables, there are many registered pesticides MRLs are now available on countries database worldwide, meaning that there is a general lack of MRLs authorized in Ethiopia regulations, owing to the lack of studies on residue trials necessary for registration of pesticides and the establishing of MRLs [23].

For the pesticides evaluated, no MRL was found for dibutyl chlorendate and chloroflutenol - methyl. Due to this, in some cases, MRLs are set by default at a specific low value, even lower than the LOD of analytical methods developed for pesticides in matrices. A general default MRL of 0.01 mg/kg applies where a pesticide is not specifically mentioned in EU legislation [84]. Considering this MRL, all the samples contained dibutyl chlorendate residue exceeding the default MRL. Therefore, the detection of this chemical by this level in all sample indicates, maybe there is a misuse of agrochemicals or historical use of these chemicals among sampling area farmers [9, 43]. This intensive use of pesticides in horticultural crops without observation of good agriculture practices and regulations has caused great concern with a probable final product contamination [83]. When compared with a recent study done in Malaysia [85], Ghana [80] and Egypt [81] in all tomato, potato and pineapple sample under this study contained a lower concentration of all OCPs.

Additionally, all the samples included more than one pesticide; the reason being that tomato, potato, and pineapple cultivated under some conditions is highly sensitive to pests and requires successive applications of different pesticide treatments [86]. Even if, applications of chemical synthetic pesticides are not allowed in organic agriculture, there can never be a guarantee that organically grown crops are completely pesticide-free, means that risk of cross-contamination cannot be excluded [87]. In view of this, despite the remarkable economic and agricultural benefits of pesticides, they are a reason for popular concern as a result of their likely harmful results on human safety. Therefore, they need to be properly used according to Good Agricultural Practices (GAP) [88].

Good agricultural practices in the use of pesticides are the officially recommended or authorized use of pesticides under practical conditions at any stage of production, storage, transport, distribution, and processing of food and other agricultural commodities, although there are variations in requirements within and between regions and minimum quantities necessary to achieve adequate control. Pesticides being applied in such a manner leave residues in the smallest amount, so as not to cause harm to humans or animals during their lifetime [89]. Furthermore, MRLs are not safety levels but indicates legal issues in relation to pesticide use such as illegal use of obsolete or banned pesticides; the use of sub-standard formulations; or contamination from various sources including uses to protect public health, etc [43].

4.3 Comparison of pesticide residue in fruit and vegetable with other reported result

The comparisons of obtained result with other reported result were made based on mean concentration in terms of nano-gram per gram (ng/g) in tomato potato and pineapple.

Table 7 Residue levels of pesticides in other countries compared with Ethiopia

Country	Pesticides	Conc. (ng/g)	Food items	References
Bangladesh	Chlorpyrifos	342.00*	Tomato	[26]
Malaysia	Dieldrin,	ND	Tomato	[85]
	Endrin	ND		
Ghana	Methoxychlor	4.00		
	Dieldrin	4.00		
	Endrin	ND		
	p,p'-DDE	13.00	Tomato	[80]
	p,p'-DDT	12.00		
	Dimethoate	13.00*		
	Chloropyrifos	26.00*		
	Malathion	38.00*		
Egypt	Chlorpyrifos	40.00*	Potato	[81]
	p,p'- DDE	9.00		
Bolivia	Chlorpyrifos	730.00*	Potato	[82]
Ghana	Malathion	6.00		[80]
	Methoxychlor	31.00*		
	Dieldrin	12.00*		
	Endrin	4.00		
	p,p'-DDE	ND	Pineapple	
	Dimethoate	6.00		
	Chloropyrifos	55.00*		
	p,p'-DDT	ND		
Ethiopia	Dimethoate	6.37 - 58.74*		
	Malathion	18.11*	Tomato,	
	Chloropyrifos	1.73 - 2.97	Potato	Present study
	p,p'-DDE	1.13 - 1.93	& Pineapple	
	p,p'-DDT	ND		
	Endrin	ND		
	Endosulfan sulfate	0.94 - 2.21		
	Dieldrin	ND		
	Methoxychlor	1.71 - 8.78		

Values designated by asterisks are higher than the EU MRLs for the respective pesticides.

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

These findings suggest that all the samples of tomato, potato, and pineapple contained residues of six or more active substances. From this active substances, dibutyl chlorendate and chloroflurenol-methyl compounds were exceeded the EU default maximum residue limits in all the samples. Hence, the contamination levels of these residues may be considered a serious public health problem according to the maximum residue limits (MRLs) of EU and CAC and this indicates to an urgent need to develop comprehensive intervention measures to reduce the potential health risk to consumers. Some of the compounds such as p,p'- DDT, endrin, dieldrin, dimethoate (except Saka buyo qacama and Mana) and malathion (except Saka buyo qacama) which were not detected at all. Other active compounds such as chloropyrifos, endosulfan sulfate, DDE and methoxychlor in all analyzed samples showed low levels of pesticide residues, near the limits of quantification (LOQ). Although, these residues were detected in very small amounts does not mean that their presence in the fruits and vegetables should just be ignored. These pesticides have the potential to affect human health and therefore we should be concerned and address the issue appropriately.

5.2 Recommendations

Based on the findings of the study, the following recommendations are suggested:

- Use of OP and OC pesticides is a habitual practice in fruit and vegetable production in Ethiopia, hence monitoring of this chemicals should be done at regular interval to determine the extent of the release of this compound to the environment and food products.
- It is necessary to develop appropriate control, monitoring, and management strategies on pesticide use by the authorized body for the purpose of organic crops authentication.
- There is a need for extensive studies on residue trials necessary for registration of pesticides to establish pesticide MRLs database.
- It is better to do an extensive study on the health risk assessment of the detected pesticides.
- It is better to adopt good agriculture practices or integrated pest management (IPM) in Ethiopian farmers to produce non-contaminated fruit and vegetable crops.
- In addition, as the government has given a great concern to this pesticide residue it is better to work further investigation on fruit and vegetable to prevent any health problem to consumers
- Consequently, there is a need for extensive studies on pesticide residue of dibutyl chlorendate and chloroflurenol-methyl in tomato, potato, pineapple and other crop samples in all the districts, in order to determine the major source and it's concentration level.

6. REFERENCES

1. Daba D., Hymete A., Bekhit A.A, Mohamed A.M, Bekhit Ael-D: Multi-residue analysis of pesticides in wheat and khat collected from different regions of Ethiopia, *bulletin of environmental contamination and toxicology*, **2011**, 86, 336 - 341.
2. Belay T. M: Environmental governance of pesticides in Ethiopian vegetable and cut flower production, *PhD* dissertation, *Wageningen University*, **2016** DOI: http://dx.doi.org/10.18174/391632,
3. Amadeo R. Fernandez-A, Juan F and Garcı´a-R: Large-scale multi-residue methods for pesticides and their degradation products in food by advanced LC-MS, *trends in analytical chemistry*, **2008**, 27 (11), 973 - 990.
4. Maurizio G. P and David P: Environmental risks of pesticides versus genetic engineering for agricultural pest control, *Journal of agricultural and environmental ethics*, **2000**, 12**,** 279 - 303.
5. Maria Helena W. M. C., Adherlene Vieira G., Armi Wanderley of N cheesy and Shirley de Mello P. A: Extraction and clean-up method for the determination of twenty organochlorine pesticide residues in tomatoes by GC-ECD, *journal of high resolution chromatography*, **1999**, 22, 619 - 622.
6. Jolanta F., Maciej T and Marek B: Properties and determination of pesticides in fruits and vegetables, *trends in analytical chemistry*, **2011**, 30, 814 - 827.
7. Mekonnen Y. and Agonafir T: Pesticide sprayers' knowledge, attitude and practice of pesticide use on agricultural farms of Ethiopia, *society of occupational medicine*, **2002**, 52, 311 - 315.
8. Bekele D., Belyhun Y., Petros B., Deressa W: Assessment of the effect of insecticide-treated nets and indoor residual spraying for malaria control in three rural kebeles of Adami Tulu district, South Central Ethiopia, *malaria journal*, **2012**, 11(127).
9. Seblework M., Ambelu A. and Pieter S: Pesticide residue evaluation in major staple food items of Ethiopia using the QuEChERS method: a case study from the Jimma zone; *environmental toxicology and chemistry*, **2014**, 33(6), 1294 - 1302.
10. Tadesse A. and Asferachew A: An assessment of the pesticide use, practice, and hazards in the Ethiopian rift valley, Annex 6 for Africa stockpiles program, **2008**.

11. Crentsil Kofi B., Asomaningl's J and Juliana B: Market basket survey for some pesticides residues in fruits and vegetables from Ghana, *journal of microbiology, biotechnology and food sciences,* **2012**, 2(3), 850 - 871.
12. Ngan, C. K., Khairatul, A. M., Shahid, S. M and Ismail, B. S: Validation of a 'multi-residue' analytical method for pesticide residue analysis in fruits, *journal of tropical agriculture and food science*, **2013**, 41(1), 119 - 126.
13. Cervera M. I., Medina C., Portolés T., Pitarch E., Beltrán J., Serrahima E., Pineda L. Muñoz G., Centrich F and Hernández F: Multi-residue determination of 130 multiclass pesticides in fruits and vegetables by gas chromatography coupled to triple quadrupole tandem mass spectrometry, *analytical and bio-analytical chemistry* **2010**, 397, 2873 - 2891.
14. Paula P., Michelangelo A., Dorothea M., Irina S., Bünyamin T., José O and Alberto B: Analysis of pesticide residues using the Quick Easy Cheap Effective Rugged and Safe (QuEChERS) pesticide multi-residue method in combination with gas and liquid chromatography and tandem mass spectrometric detection, *analytical and bio-analytical chemistry*, **2007**, 389, 1697-1714.
15. Ministry of agriculture: An economic review of agriculture on the central planning and project monitoring unit, Kilimo house cathedral road, Nairobi, Kenya, **2008**.
16. The current population of **Ethiopia** based on the latest United Nations estimates.downloadable at http://www.worldometers.info/world-population/Ethiopia-pop-n (Accessed on 03/10/2018).
17. Agonafir Y: Economics of horticultural production in Ethiopia https://doi.org/10.17660/ www.actahort.org, **1991** (Accessed on 03/10/2018).
18. Mengistie B. T: Policy-practice Nexus: pesticide registration, distribution and use in Ethiopia. SM, *journal of environmental toxicology*, **2016**, 2(1), 1006.
19. Emana D., Fekadu D., Victor A-S and Amsalu A: Characterization and assessment of vegetable production and marketing systems in the humid tropics of Ethiopia, *journal of international agriculture,* **2015**, 54 (2), 163 - 188.
20. FAO (Food and Agriculture Organization): Agroclimatic resource inventory for land use planning in Ethiopia, technical report 2. AG: DP/ETH/78/003. Rome, Italy, **1984**.

21. Hunde N. F: Opportunity, problems and production status of vegetables in Ethiopia: a review, *journal of plant science & research*, **2017**, 4(2), 172.
22. Ethiopian revenue and customs authority report of 2013 and export of **2013**, Addis Ababa; downloadable at http://www.erca.go.et (Accessed on 03/10/2018).
23. Ashenafi G., Berhanu T and Retta N: A thesis project on the analysis of organochlorine pesticide residues in orange and tomato samples from piassa Atekelt tera, Addis Ababa; *journal of Addis Ababa university*, **2017** (Accessed on 03/10/2018).
24. Soma S. and Kalpana K: Nutritional content and significance of tomato powder, *Annals of arid zone*, **2013**, 52(2), 121 - 124.
25. Tendai M.W., Leia W., Cara H and John W. Z: Nutritional and health benefits of fresh vegetables - past, present, and future, *a literature review*, **2007**.
26. Fardous Z., Islam M. N., Hoque S. M., Choudhury M. A. Z and Rahman M. A: Determination of some selected pesticide residues in tomato from different locations of Bangladesh, *international journal of sustainable agricultural technology*, **2007**, 3(6), 04 - 07.
27. Tarsicio Medina S., Gabriela Arroyo F and Jorge Gustavo D. C: Origin and evolution of tomato production Lycopersicon esculentum in México; Ciência Rural, Santa Maria, **2017**, 47(3).
28. Ambecha O., Gemechis, Paul C. S and Bezabih E: Tomato production in Ethiopia: constraints and opportunities, **2015**; downloadable at http://www. researchgate.net/publication/265999979 (Accessed on 03/10/2018).
29. Tesfa B., Yosef A., Jibicho G., Gebeyehu W and Melkamu H: Performance of introduced hybrid tomato (Solanum lycopersicum Mill.) cultivars in the rift valley, Ethiopia; *journal of research in agriculture and forestry*, **2016**, 3(10), 25 - 28.
30. Sanjiv A. and Akkinappally V. Rao**:** Tomato lycopene and its role in human health and chronic diseases, *Canadian Medical Association or its licensors*, **2000**, 163(6), 739-744.
31. Danielle D and Stan K.: Role of potato in human health, **2011,** downloadable at https://www.agrireseau.net/pdt/documents/donnelly_kubow.pdf (Accessed on 03/10/2018).

32. Hailu G., Ali M., Nigussie D and Derbew B: Assessment of production practices of smallholder potato (*Solanum tuberosum* L.) farmers in Wolaita zone, southern Ethiopia; *agriculture & food security*, **2017**, 6(31).
33. Asresie H., Alemu W., Molla T., Mekonen T., Abel A., Seferew D., Yihenew G. S., Desallegn M. and Tesfaye A: Best fit practice manual for potato production and utilization: BDU-CASCAPE working paper 8, **2015**.
34. Leila M. B. R., Kamilla de L. R., Maria Eliana L. R. de Q., Antônio A. N., Laércio Z and Ricardo M. O: Determination of chlorpyrifos and thiamethoxam in potato tuber (*Solanum tuberosum L.*) and soil of Brazil using solid-liquid extraction with low temperature partitioning (SLE/LTP); *journal of the Brazilian chemical society*, **2013**, 24 (12), 2042 - 2049.
35. Helena B. Č., Špela velikonja B., and Ana .G: Pesticide residues in samples of apples, lettuce and potatoes from integrated pest management in Slovenia from 2005-2009, **2012**, 99(1), 49 - 56.
36. Umadevi M., Sampath Kumar P. K., Debjit Bhowmik, Duraive S: Health benefits and cons of *Solanum tuberosum, journal of medicinal plants studies*; **2013**, 1(1), 16 - 25.
37. Orluchukwu J. A and Adedokun O. M: Response of growth and yield of pineapple (*Ananas comosus)* on spent mushroom substrates and inorganic fertilizer in South-South, Nigeria; *journal of plant & soil science*, **2015**, 8(6), 1 - 5.
38. Isiaka Baruwa O: Profitability and constraints of pineapple production in Osun State, Nigeria; *journal of horticultural research*, **2013**, 21(2), 59 - 64.
39. Ester Alice F., Heloisa Elias S., Eduardo Valerio V. B., Vanessa Stahl H., Alessandro De O. R.: Bioactive compounds and antioxidant activity of pineapple fruit of different cultivars; *revista brasileira de fruticultura jaboticabal - SP*, **2016**, 38(3), e-146.
40. Ubi W., Iqwe H., Ekpe S., Ibor O: Preliminary trial of fertilizer types on pineapple (*Ananas comosus*) grown on coastal and sands of cross river state, Nigeria; *global journal of pure and applied sciences*, **2005**, 11(4), 457-460. DOI: 10.4314/gjpas.v11i4.16534.

41. Pineapple juice health: http://livewell.jillianmichaels.com/acids-pineapple-juice-5081.html (Accessed on 03/10/2018).
42. Farid Hossain Md., Shaheen A., Mustafa A: Nutritional value and medicinal benefits of pineapple, *journal of nutrition and food sciences*, **2015**, 4(1), 84 - 88.
43. Seblework M., Argaw A., Belay N and Pieter S: Exposure to DDT and its metabolites from khat (Catha edulis) chewing: *regulatory toxicology and pharmacology*, **2017**, 87, 64 - 70.
44. Lucio G. C., Gennaro G., Marina G., Annabella.V: Neurotoxicity of pesticides; a brief review, *frontiers in bioscience*, **2008**, 13, 1240 - 1249.
45. De Silva H. J., Samarawickrema N. A., and Wickremasinghe A. R: Toxicity due to organophosphorus compounds: what about chronic exposure, transactions of the royal society of tropical medicine and hygiene, **2006**, 100, 803 - 806.
46. Kaushik, J. and Chandrabhan D: Sources of exposure to and public health implications of organophosphate pesticides *revista panamericana de salud pública/pan american journal of public health*, **2003**, 14(3).
47. Somayyeh K. M. and Mohammad A: Toxic influence of organophosphate, carbamate, and organochlorine pesticides on cellular metabolism of lipids, proteins, and carbohydrates: *a systematic review, human and experimental toxicology*, **2010**, 30(9), 1119 - 1140.
48. Francisco P. G., Sandra Y. Cortés A., John C. Gaytan O., Alejandra C. H and Patricia Vazquez A: Pesticides, classification, uses, and toxicity; measures of exposure and genotoxic risks, *journal of research in environmental science and toxicology* (ISSN: 2315-5698), **2012**, 1(11), 279 - 293.
49. Osman T and Cemile T: The fate of pesticide in the environment, *journal of biodiversity and environmental sciences*, **2010**, 4(10), 29 - 38.
50. Racke K. D., Skidmore M. W., Hamilton D. J., Unsworth J. B., Miyamoto J., and Cohen S. Z: Pesticide fate in tropical soils (technical report), international union of pure and applied chemistry, **1997**.
51. Holger C., Julia B., Wulf A., Suphot T and Martin K: Insecticide dissipation after repeated field application to a northern Thailand Ultisol, *journal of agricultural and food chemistry*, **2006**.

52. Zorawar S., Jasminder K., Ravneet K and Swarndeep S. H: Toxic effects of organochlorine pesticides; a review, *American journal of bioscience*, **2016**; 4(3-1), 11 - 18.
53. Shemsu L: Assessments of pesticide use and practice in Bulehora districts of Ethiopia, *the Saudi journal of life sciences*, **2016**.
54. Małgorzata R., Kinga D., Piotr K., and Jacek N: Micro-extraction techniques used in the procedures for determining organo-mercury and organo-tin compounds in environmental samples; ISSN 1420-3049 www.mdpi.com/journal/molecules, **2014**, 7581 - 7609.
55. Guan H. T and Lukman B. A: Recent developments and applications of micro-extraction techniques for the analysis of pesticide residues in fruits and vegetables, http://dx.doi.org/10.5772/46843, **2012**.
56. Kapil K: Method development and validation of analytical procedures, www.intechopen.com, **2012**.
57. Ashish C., Bharti M. and Priyanka C: Analytical method development and validation: a concise review, *journal of analytical and bio-analytical techniques* 6: 233, **2015**, ISSN: 2155-9872 JABT at doi: 10.4172/2155-9872.1000233.
58. Lambropoulou D. A and Albanis, T. A: Methods of sample preparation for determination of pesticide residues in food matrices by chromatography - mass spectrometry - based techniques : a review, **2007**, 389(6), 1663 - 1683 DOI: 10.1007/1301 -1348.
59. Narenderan S. T and Meyyanathan S. N: Sample treatment and determination of pesticide residues in potato matrices: *a review*, potato research at https://doi.org/10.1007/s11540-018-9396-x, **2018**.
60. Ligani S and Hussen A: Determination of organochlorine pesticide residue levels in chewable parts of the Khat (Catha edulis) Plant, *Bull Environ Contam Toxicol*, **2014**, 93:591–595
61. WHO, the public health impact of pesticides used in agriculture, **1990.**
62. Dasika R., Tangirala S and Naishadham P: Pesticide residue analysis of fruits and vegetables, **2012**, 4(2), 19 - 28.

63. Sadowska-rociek A., Manuel J., Ruiz M and Surma-Zadora M: Evaluation of QuEChERS method for the determination of organochlorine pesticide residues in selected groups of fruits, **2011**, 125, 773 - 778.
64. Rejczak T., Tuzimski T and Access O: A review of recent developments and trends in the QuEChERS sample preparation approach, **2015**, 980 - 1010.
65. Chai L., Zaidel N., Christian H and Hansen, B: A rapid multi-residue method for the determination of pesticide residues in Choi sum, yardlong beans, and aubergines, *food chemistry*, **2012**, 131(2), 611 - 616.
66. Pérez-Ortega P., Lara-Ortega F. J and Gilbert - López, B. et al: Screening of over 600 pesticides, veterinary drugs, food-packaging contaminants, mycotoxins, and other chemicals in food by ultra-high performance liquid chromatography quadrupole time-of-flight mass spectrometry (UHPLC-QTOFMS), journal of f*ood analytical methods*, **2017,** 10, 1216.
67. Sharma D., Nagpal A., Pakade Y. B and Kaur J: Talanta analytical methods for estimation of organophosphorus pesticide residues in fruits and vegetables; a review, *Talanta***, 2010**, 82(4), 1077 - 1089.
68. Jin B., Xie L., Guo Y and Pang G: Multi-residue detection of pesticides in juice and fruit wine ; a review of extraction and detection methods. *FRIN*, **2012**, 46(1), 399 - 409.
69. The codex alimentarius commission general guidelines on sampling (FAO/WHO, **2014**) downloadable at http://www.fao.org/uploads/media/Codex 2004 sampling CAC GL 50.pdf (Accessed on 03/10/2018).
70. Lehotay S. J: Determination of pesticide residues in food by acetonitrile extraction and partitioning with magnesium sulfate: Collaborative study, *journal of AOAC international*, **2007**, 90(2), 485 - 521.
71. Lavanya G, Sunil M, Eswarudu M. M., Chinna E. M., Harisudha K, and Naga Spandana B: Analytical method validation: an updated review**,** *international journal of pharmaceutical sciences and research,* **2013**, 4(4), 1280 - 1286.
72. Hajslova J., Holadova K., Kocourek V., Poustka J., Godula M., Cuhra P and Kempn M; Matrix-induced effects: A critical point in the gas chromatographic analysis of pesticide residues, *journal of chromatography A*, **1998**, 800, 283 - 295.

73. Marina R., Svetlana G., Tatjana V and Mila L: Determination of selected pesticides in fruit juices by matrix solid-phase dispersion and liquid chromatography-tandem mass spectrometry, *journal of food chemistry*, **2009**, 113, 712 – 719.
74. Yang X., Zhang H., Liu Y., Wangc J., Zhang Y. C., Dong A. J., Zhao H. T., Sun C. H and Cui J: Multi-residue method for determination of 88 pesticides in berry fruits using solid-phase extraction and gas chromatography-mass spectrometry, *food chemistry*, **2011**, 127, 855 – 865.
75. European commission method validation and quality control procedures for pesticide residues analysis in food and feed, at the document number; SANCO/10684/2009, Brussels, ec.europa.eu/food/plant/protection/resources/qualcontrolen.pdf, **2010**.
76. EU pesticides database: downloadable athttp://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=pesticide.residue.selection&language=EN (Accessed on 03/10/2018).
77. Codex alimentarius commission pesticide database: downloadable at http://www.fao.org/fao-who-codexalimentarius/codex texts/dbs/pestres/pesticides/en/ (Accessed on 03/10/2018).
78. Guan-Soon, L: Overview of vegetable IPM in Asia. *FAO plant proc. Bull. Indian Publication at* 1974, **1990**, 23(3), 25 - 28.
79. Hernández-Borge J., Cabrera J. C., Rodríguez-Delgado M. A., Estrella M. H. S and Saúco V. G: Analysis of pesticide residues in bananas harvested in the Canary Islands (Spain), *journal of food chemistry*, **2009**, 113, 313 - 319.
80. Bempah C. K., Asomaning J., Ansong D.A., Boateng J and Asabere S. B: Contamination levels of selected organochlorine and organophosphorus pesticides in Ghanaian fruits and vegetables, *Emir journal of food and agriculture*, **2012**, 24 (4), 293 - 301.
81. Mohamed A.I. A., Nasr S. K. and Tarek A. A. E.R: Determination of pesticide residues in potato tuber samples using QuEChERS method with gas chromatography; *Australian journal of basic and applied sciences*, **2014**, 8(3), 349 - 353.

82. Skovgaard M., Renjel S., Jensen O. C., Andersen J. H., Condarco G and Jors E: Pesticide residues in commercial lettuce, onion, and potato samples from Bolivia - a threat to public health? *Environmental health insights*, **2017**, 11(0), 1 - 8. DOI: 10.1177/1178630217704194.
83. Amera T and Abate A: An assessment of the pesticide use, practice and hazards in the Ethiopian rift valley. Africa Stockpiles programme, available from: http://www.thenrgroup.net/theme/PAN-ecotox/pdf/annex_6_ethiopia_mini-project_report.pdf (Accessed on 05/10/2018), **2008**.
84. Botero-Coy A. M., Marin J. M., Serrano R., Sancho J. V and Hernandez F: Exploring matrix effects in liquid chromatography-tandem mass spectrometry determination of pesticide residues in tropical fruits, *analytical and bio-analytical chemistry*, **2015**, 407, 3667 - 3681.
85. Zawiyah S., Che Man Y. B., Nazimah S. A. H., Chin C. K., Tsukamoto d I., Hamanyza A.H and Norhaizan I: Determination of organochlorine and pyrethroid pesticides in fruit and vegetables using SAX/PSA clean-up column, *food chemistry,* **2007,** 102, 98 - 103.
86. Jamshid S., Attaollah S and Vahideh M: Occurrence of multiclass pesticide residues in tomato samples collected from different markets of Iran, *journal of environmental health science and engineering*, **2018**, downloadable at https://doi.org/10.1007/s40201-018-0296-4 (Accessed on 05/10/2018).
87. MacLachlan D. J., and Hamilton D: Estimation methods for maximum residue limits for pesticides, *regulatory toxicology and pharmacology*, **2010**, 58, 208 - 218.
88. Hurst P., Alistair H and Nigel D: The pesticide handbook. Journeyman Press, London, Concord, MA, USA, **1991**.
89. FAO/WHO (Food and Agricultural Organisation/ World Health Organisation). Pesticide residues in food, report of the 1974 joint meeting of the FAO working party of experts on pesticide residues and the WHO expert committee on pesticide residues. FAO agricultural studies, No. 97. WHO technical report series, No. 574. Geneva, Switzerland, **1975**.

APPENDIX

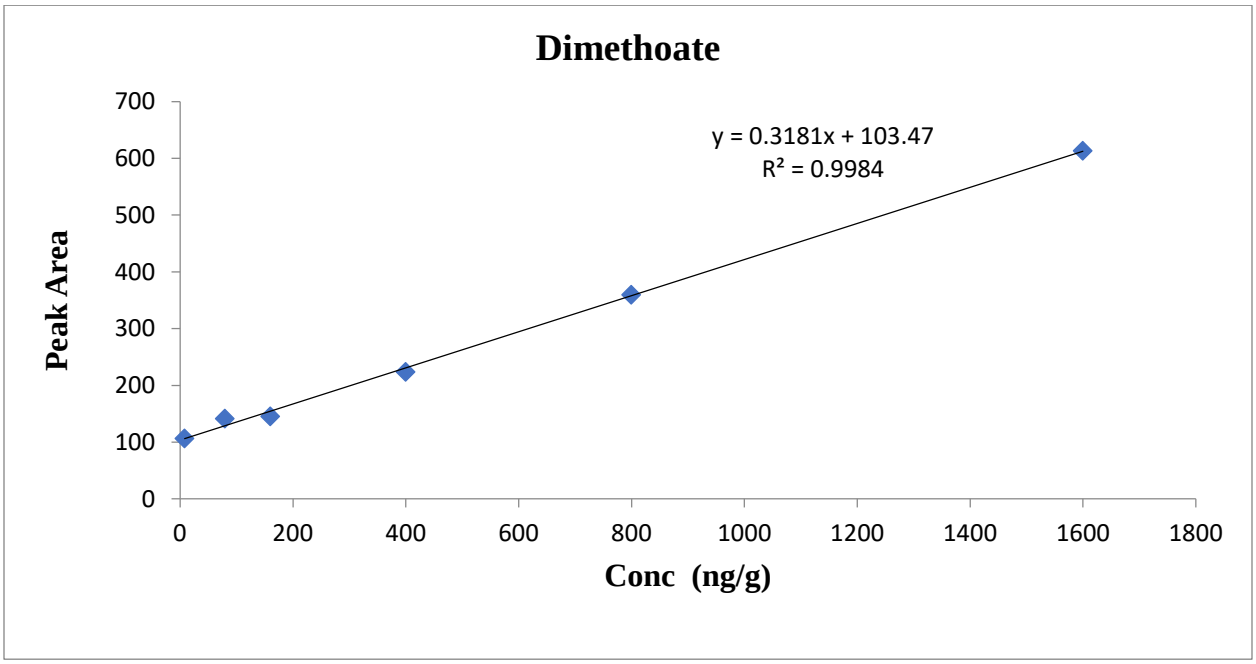
Part I: Files during sample collection and extraction time

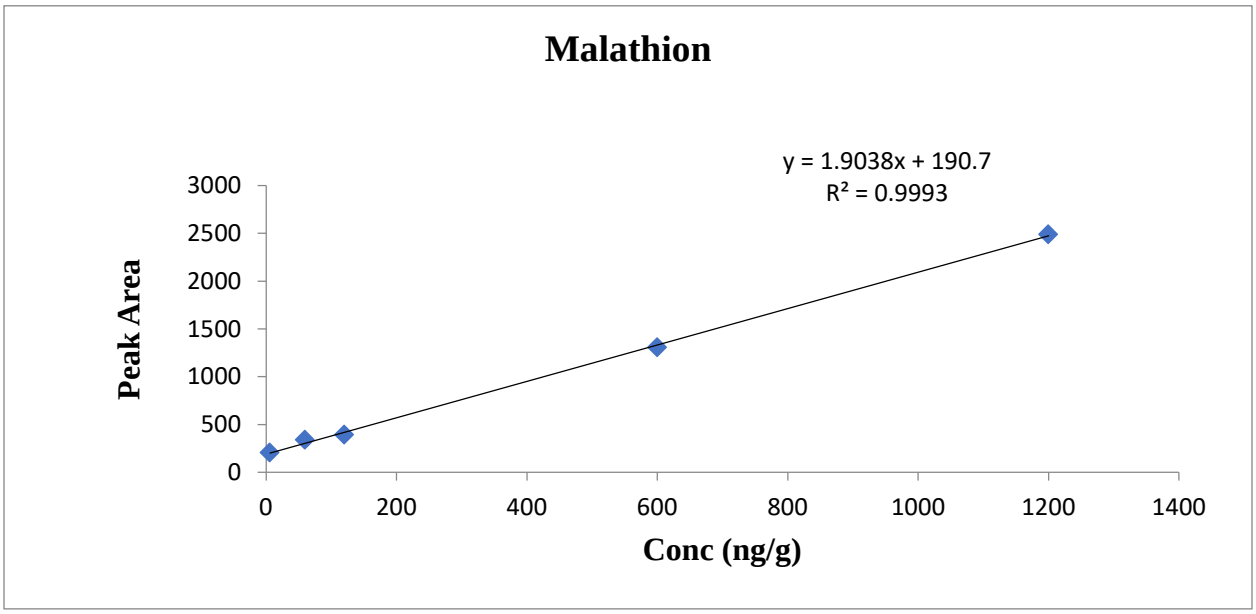
Figure 7 Sample preparation of tomato sample

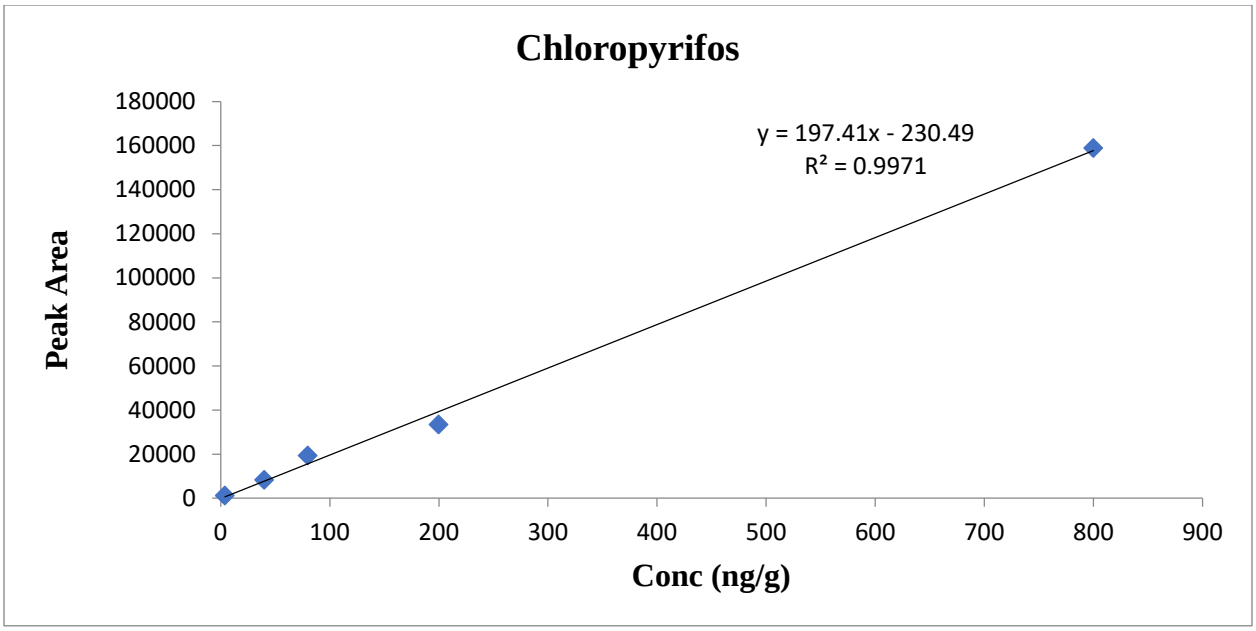
Figure 8 During sample collection of potato

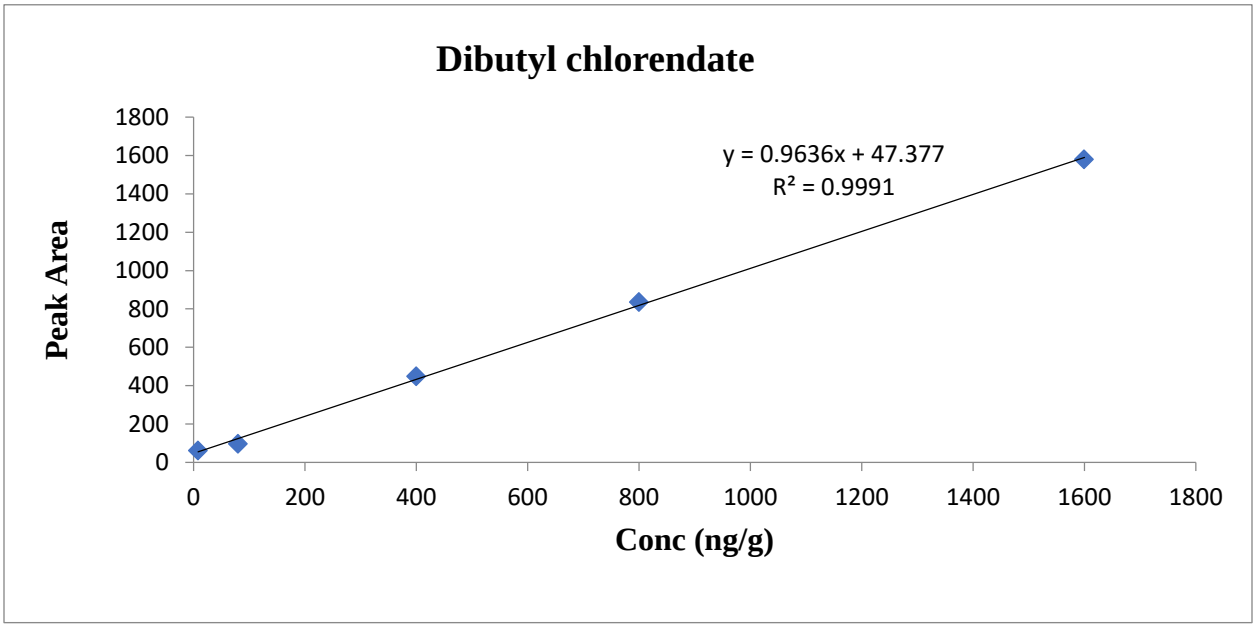
Figure 9 During the collection and preparation of pineapple samples

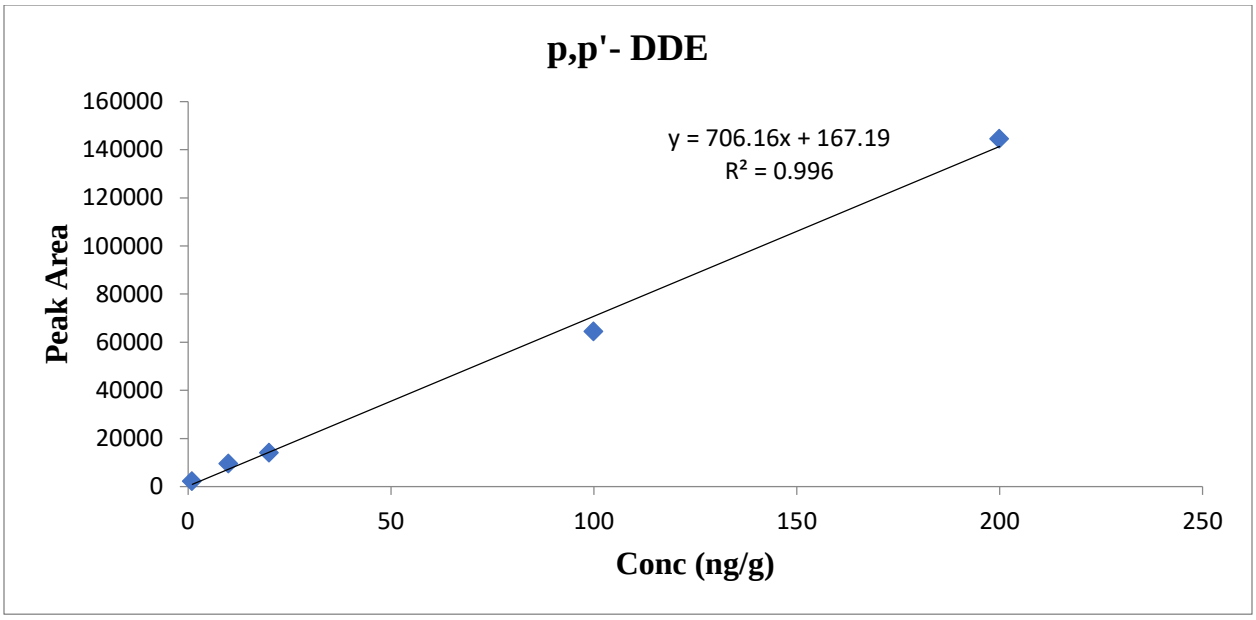
Part II: Calibration graph with their equation of each target pesticide

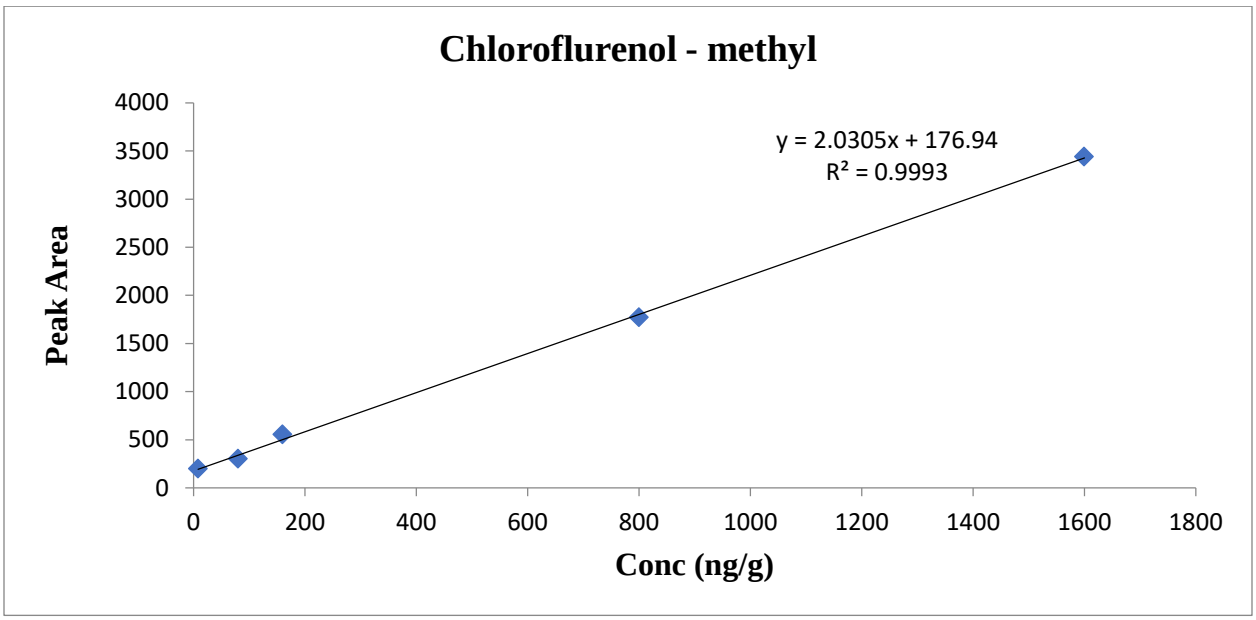


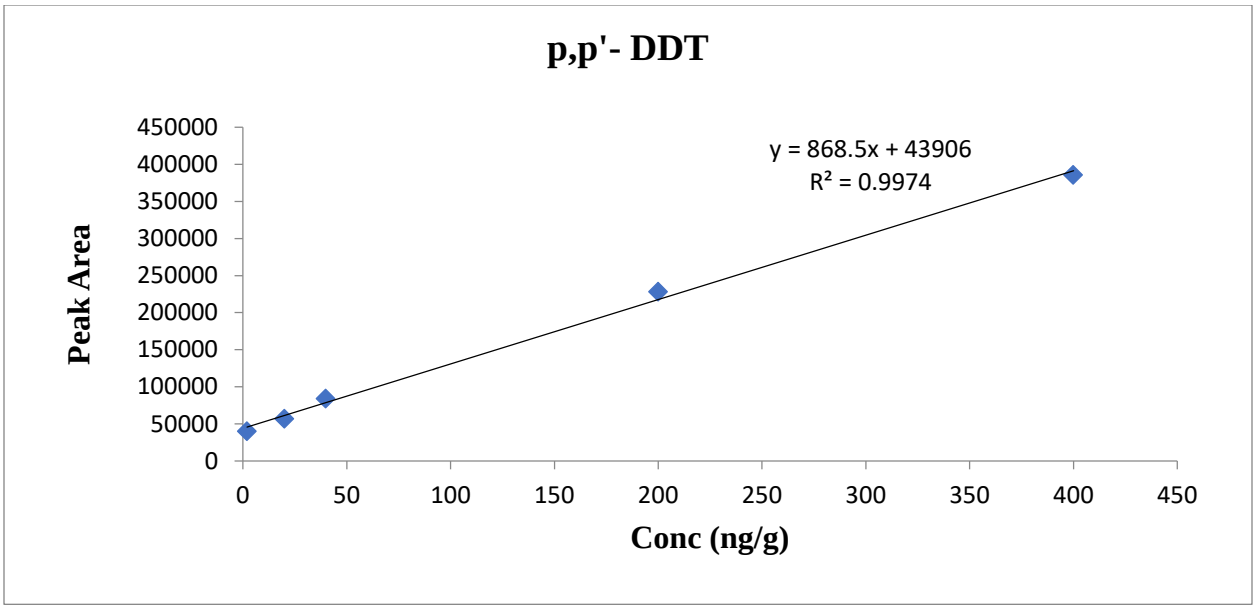


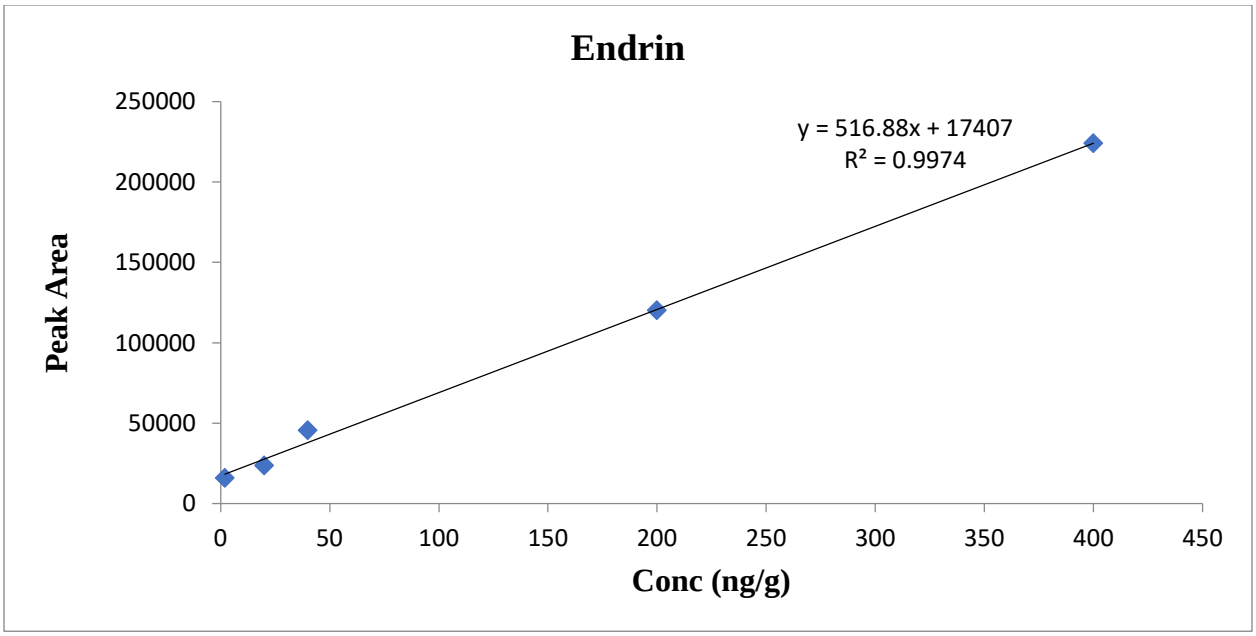

Chloropyrifos
y = 197.41x - 230.49
R² = 0.9971
Peak Area
Conc (ng/g)
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
0
100
200
300
400
500
600
700
800
900

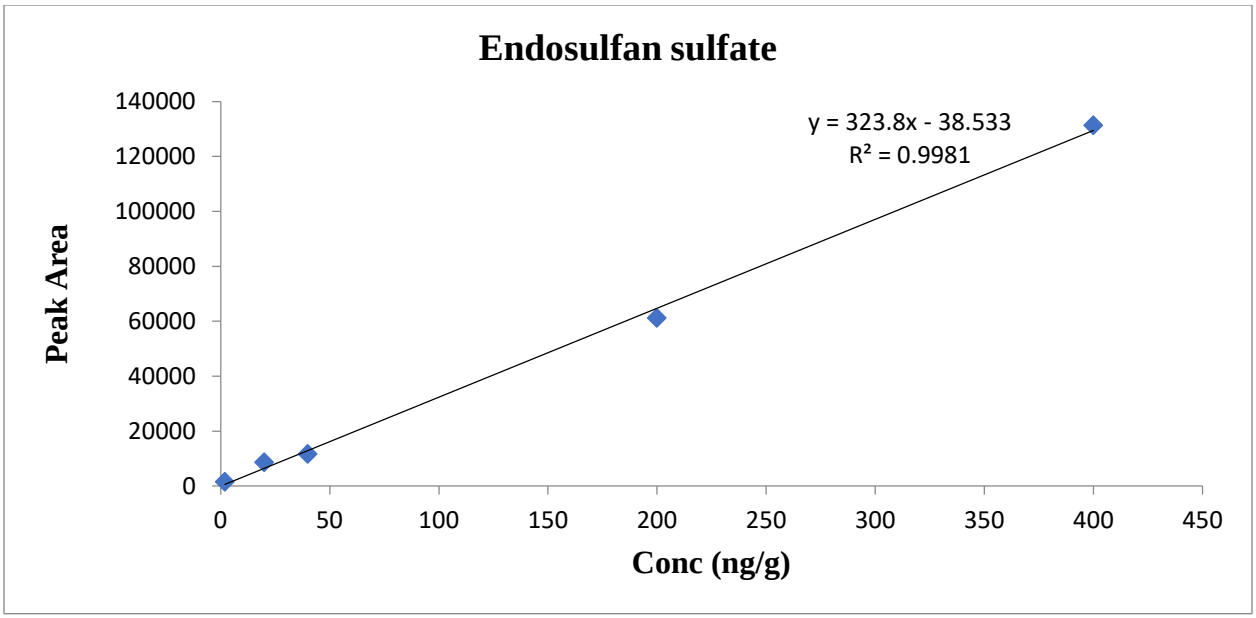

Dibutyl chlorendate
y = 0.9636x + 47.377
R² = 0.9991
Peak Area
Conc (ng/g)
1800
1600
1400
1200
1000
800
600
400
200
0
0
200
400
600
800
1000
1200
1400
1600
1800

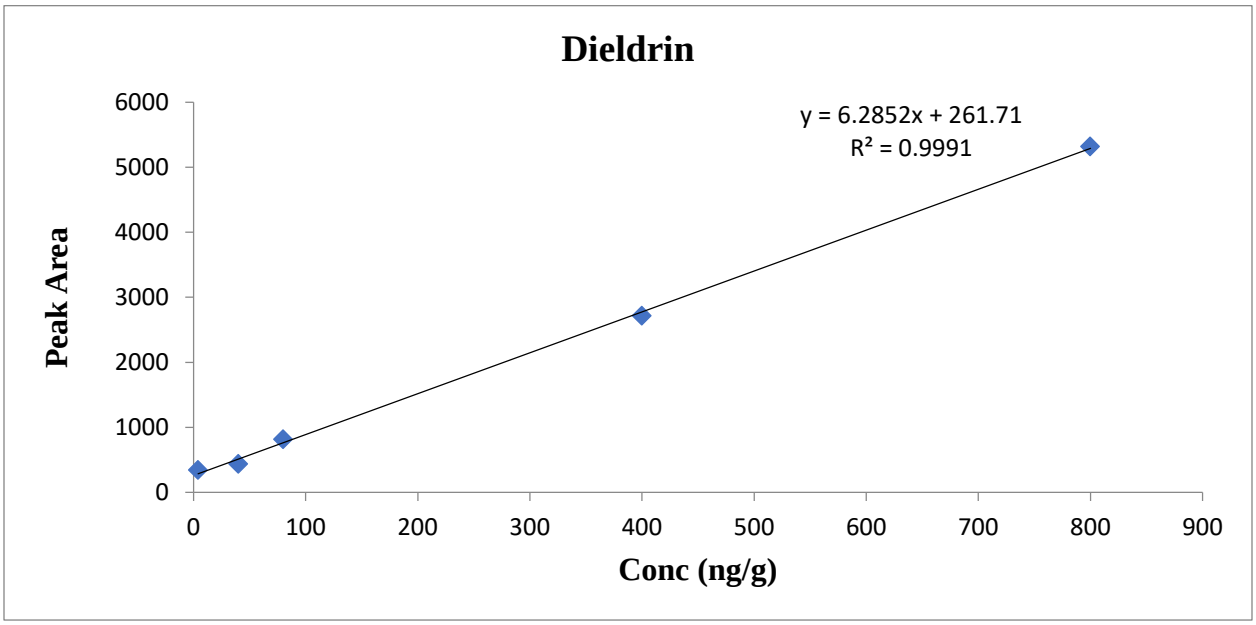

p,p'- DDE
y = 706.16x + 167.19
R² = 0.996
Peak Area
Conc (ng/g)
160000
140000
120000
100000
80000
60000
40000
20000
0
0
50
100
150
200
250


Chloroflurenol - methyl
y = 2.0305x + 176.94
R² = 0.9993
Peak Area
Conc (ng/g)
4000
3500
3000
2500
2000
1500
1000
500
0
0
200
400
600
800
1000
1200
1400
1600
1800


p,p'- DDT
y = 868.5x + 43906
R² = 0.9974
Peak Area
Conc (ng/g)
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
50
100
150
200
250
300
350
400
450


Endrin
y = 516.88x + 17407
R² = 0.9974
Peak Area
Conc (ng/g)
250000
200000
150000
100000
50000
0
0
50
100
150
200
250
300
350
400
450


Endosulfan sulfate
y = 323.8x - 38.533
R² = 0.9981
Peak Area
Conc (ng/g)
140000
120000
100000
80000
60000
40000
20000
0
0
50
100
150
200
250
300
350
400
450


Dieldrin
y = 6.2852x + 261.71
R² = 0.9991
Peak Area
Conc (ng/g)
6000
5000
4000
3000
2000
1000
0
0
100
200
300
400
500
600
700
800
900

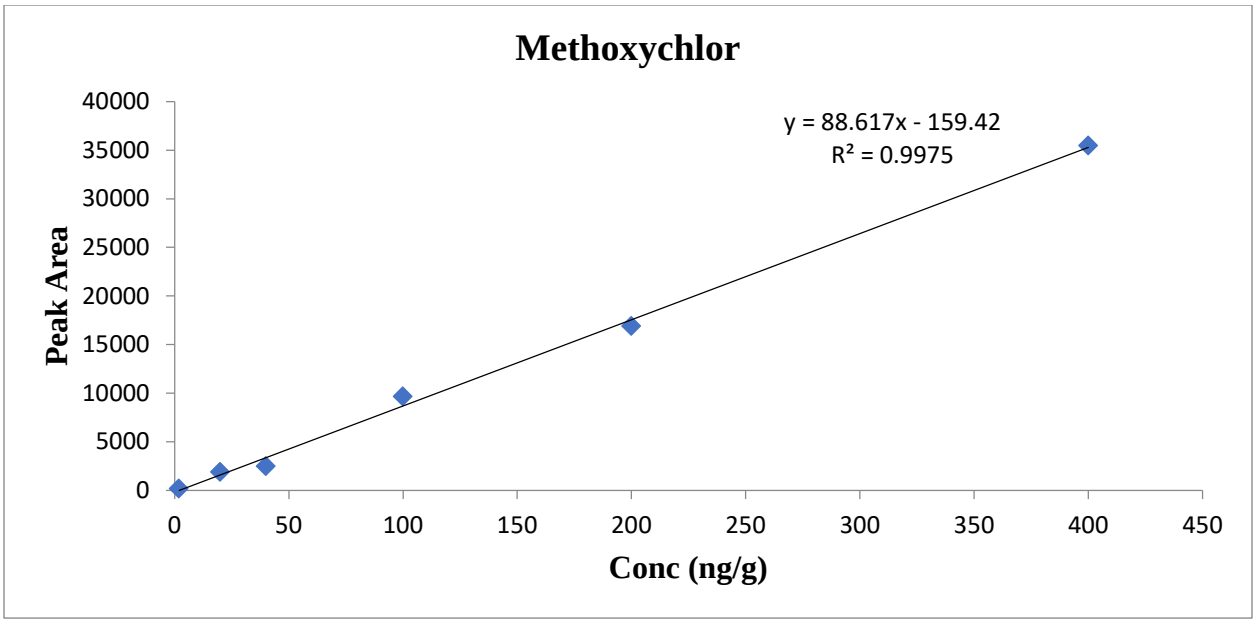


Figure 10 Calibration graphs with their equation of each target pesticide analytes

Part III: Chromatogram of the studied pesticides with their retention time (minute)

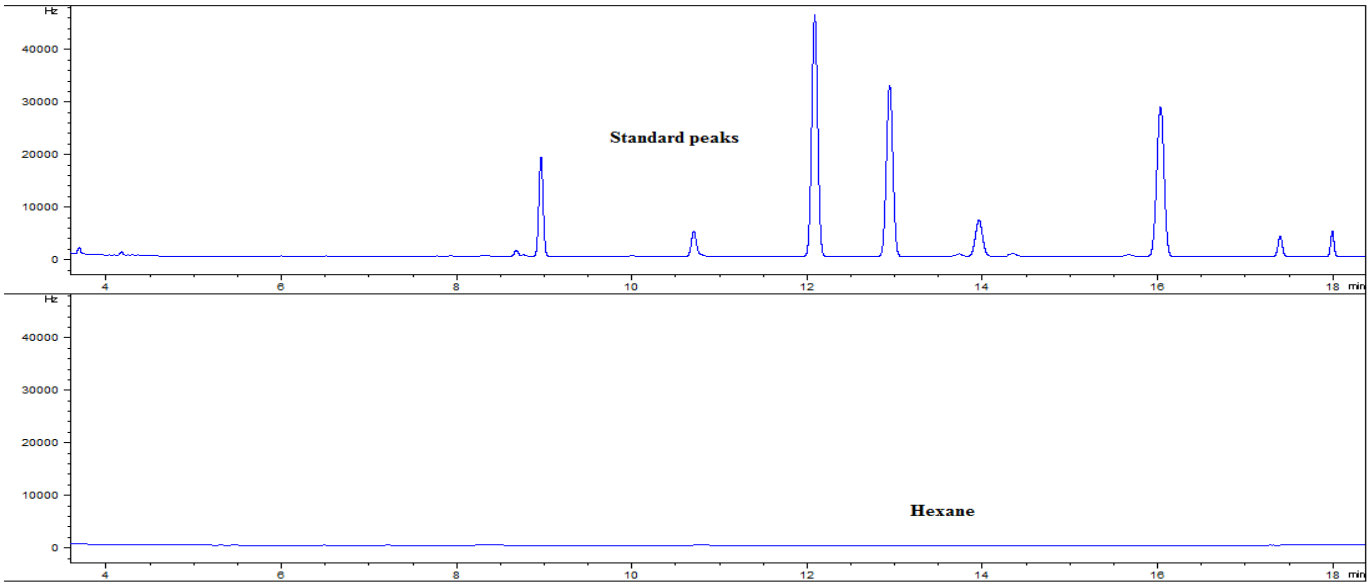


Figure 11 Chromatogram of 100 ng /mL pesticide standards with pure hexane solvent

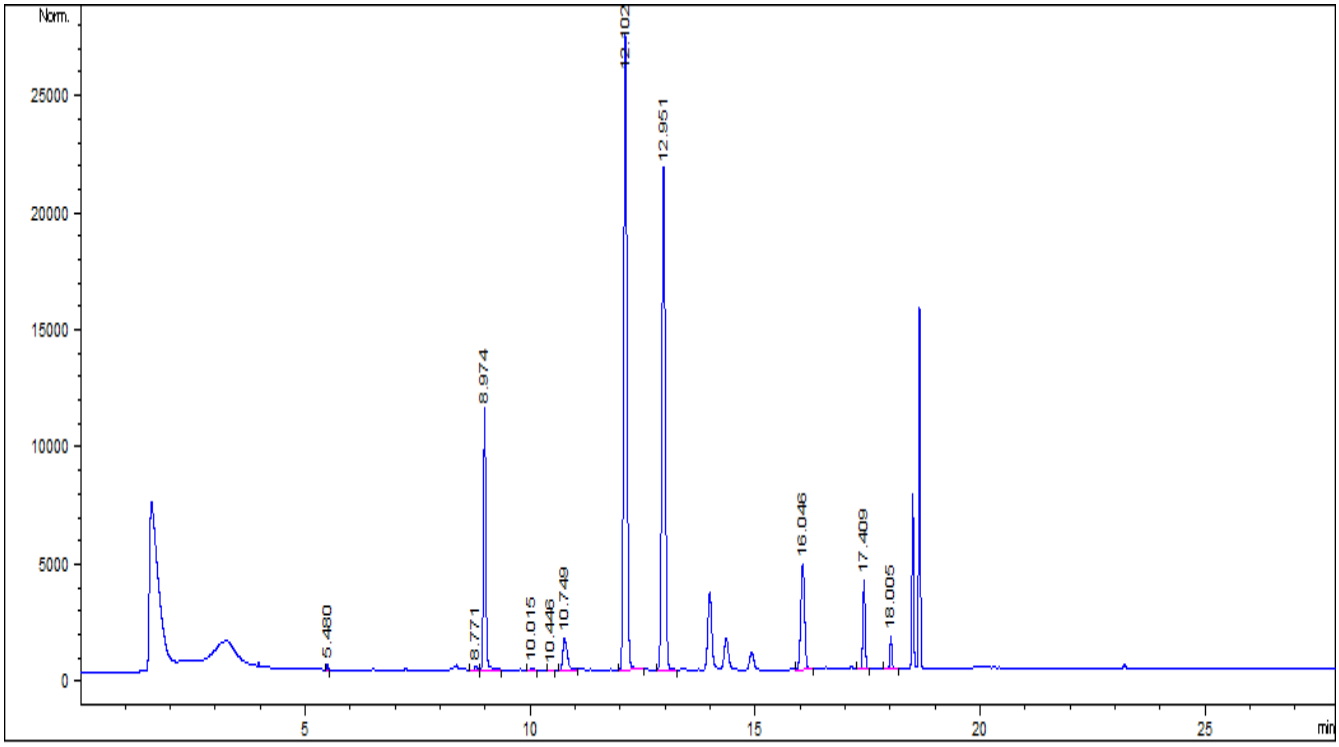


Figure 12 Chromatogram description of target analytes with their retention time, t_R, in min

Part IV: Analysis of variance table for the studied analytes

Table 8 Analysis of variance for Chloropyrifos in pineapple sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	1.93	0.48	216.83	<.0001	1.86
Replication(R)	3	0.02	0.005	2.3	0.1288	
Error(E)	12	0.03	0.002			
Corrected Total	19	1.98				

NB: *df=degree of freedom: SS=Sum of Square: MS = Mean of Square: T=Treatment: R=Replicate: E=Error: CV=Coefficient of variation*

Table 9 Analysis of variance for DDE in pineapple sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	0.81	0.20	189.22	<.0001	1.91
Replication(R)	3	0.002	0.0006	0.56	0.653	
Error(E)	12	0.01	0.001			
Corrected Total	19	0.82				

Table 10 Analysis of variance for Endosulfan sulfate in pineapple sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	2.14	0.54	80.32	<.0001	4.77
Replication(R)	3	0.03	0.01	1.35	0.304	
Error(E)	12	0.08	0.01			
Corrected Total	19	2.25				

Table 11 Analysis of variance for Methoxychlor in pineapple sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	103.77	25.94	251.14	<.0001	4.79
Replication(R)	3	0.74	0.25	2.39	0.12	
Error(E)	12	1.24	0.10			
Corrected Total	19	105.75				

Table 12 Analysis of variance for DBC in pineapple sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	3900353.37	975088.34	21882.9	<.0001	0.28
Replication(R)	3	331.57	110.52	2.48	0.111	
Error(E)	12	534.71	44.56			
Corrected Total	19	3901219.66				

Table 13 Analysis of variance for Chlorofiurenol - methyl in pineapple sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	2154661.20	538665.30	12739	<.0001	0.87
Replication(R)	3	59.05	19.69	0.47	0.7117	
Error(E)	12	507.42	42.29			
Corrected Total	19	2155227.67				

Table 14 Analysis of variance for Chloropyrifos in potato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	0.43	0.11	5.1	0.0123	7.48
Replication(R)	3	0.10	0.03	1.6	0.2402	
Error(E)	12	0.26	0.021			
Corrected Total	19	0.79				

Table 15 Analysis of variance for DDE in potato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	0.62	0.16	15.69	0.0001	7.17
Replication(R)	3	0.14	0.05	4.6	0.023	
Error(E)	12	0.12	0.01			
Corrected Total	19	0.88				

Table 16 Analysis of variance for Endosulfan sulfate in potato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	0.09	0.02	4.95	0.0136	4.65
Replication(R)	3	0.02	0.01	1.05	0.4051	
Error(E)	12	0.06	0.005			
Corrected Total	19	0.17				

Table 17 Analysis of variance for Methoxychlor in potato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	0.47	0.12	2.11	0.1428	9.34
Replication(R)	3	0.03	0.009	0.15	0.9246	
Error(E)	12	0.67	0.06			
Corrected Total	19	1.17				

Table 18 Analysis of variance for DBC in potato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	2973739.27	743434.82	11689.1	<.0001	0.33
Replication(R)	3	347.89	115.96	1.82	0.1966	
Error(E)	12	763.21	63.60			
Corrected Total	19	2974850.37				

Table 19 Analysis of variance for Chlorofiurenol - methyl in potato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	97709.35	24427.34	2769.48	<.0001	0.39
Replication(R)	3	30.17	10.06	1.14	0.3723	
Error(E)	12	105.84	8.82			
Corrected Total	19	97845.36				

Table 20 Analysis of variance for Chloropyrifos in tomato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	1.49	0.37	5.36	0.0103	9.81
Replication(R)	3	0.16	0.05	0.76	0.5383	
Error(E)	12	0.83	0.07			
Corrected Total	19	2.49				

Table 21 Analysis of variance for DDE in tomato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	0.47	0.12	8.50	0.0017	8.73
Replication(R)	3	0.07	0.02	1.70	0.2196	
Error(E)	12	0.17	0.01			
Corrected Total	19	0.71				

Table 22 Analysis of variance for Endosulfan sulfate in tomato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	1.03	0.26	14.6	0.0001	9.82
Replication(R)	3	0.009	0.003	0.18	0.91	
Error(E)	12	0.21	0.02			
Corrected Total	19	1.25				

Table 23 Analysis of variance for Methoxychlor in tomato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	5.68	1.42	176.74	<.0001	3.27
Replication(R)	3	0.09	0.03	3.83	0.0389	
Error(E)	12	0.10	0.008			
Corrected Total	19	5.87				

Table 24 Analysis of variance for DBC in tomato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	515791.51	128947.88	6033	<.0001	0.19
Replication(R)	3	45.65	15.22	0.71	0.56	
Error(E)	12	256.49	21.37			
Corrected Total	19	516093.64				

Table 25 Analysis of variance for Chlorofiurenol - methyl in tomato sample

Source of variation	df	SS	MS	F value	P value	CV
Treatment(T)	4	3974385.70	993596.43	40631.8	<.0001	0.67
Replication(R)	3	98.39	32.80	1.34	0.3073	
Error(E)	12	293.44	24.45			
Corrected Total	19	3974777.53				