ORGANOCHLORINE PESTICIDE RESIDUES IN HONEY FROM SOUTH WESTERN ETHIOPIA

BY: MOHAMMED ABA JEBEL

A THESIS PAPER SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES AND TECHNOLOGY, COLLEGE OF PUBLIC HEALTH, INSTITUTE OF HEALTH, JIMMA UNIVERSITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY

November 2017

Jimma, Ethiopia

JIMMA UNIVERSITY
INSTITUTE OF HEALTH

COLLEGE OF PUBLIC HEALTH

DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCES AND TECHNOLOGY

ORGANOCHLORINE PESTICIDE RESIDUES IN HONEY FROM SOUTH WESTERN ETHIOPIA

BY: MOHAMMED ABA JEBEL

Advisors:

Argew Ambelu (PhD, Associate Professor)

Asgdom Malu (Msc)

Seblework Mokenen (PhD, Assistant Professor)

Abera Gure (PhD, Assistant Professor)

November 2017

Jimma, Ethiopia

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature …… Date……

Members of the board of examiners

External Examiner	Signature	Date
…….	……..	……
Internal Examiner	Signature	Date
…….	……..	……
Chairperson	Signature	Date
…….	……..	……
Advisors	Signature	Date
Dr. Argew Ambelu	.....	.....
Mr. Asgdom Malu	.....	.....
Dr. Seblework Mokenen	.....	.....
Dr. Abera Gure	.....	.....

ABSTRACT

Ethiopia is ranking ninth highest honey producer in the world and the leading producer of honey and beeswax in Africa. Pesticide application in crops can contaminate soil, air, water, and the flowers from which bees collect nectar for honey production, which may cause the introduction of those toxic chemicals into the food chain, affecting human health. The main purpose of this study was analysis of organochlorine pesticide residues in honey collected from selected zone of southwest Ethiopia. An Experiments study was conducted and a total 22 samples were collected from 11 site selected in four different zones of southwest Ethiopia. Dispersive Liquid–Liquid Microextraction (DLLM) was used to extract residues from the honey samples and were investigated for the presence of nine organochlorine pesticide residues by using Gas Chromatography with ECD. Out of the total samples analyzed, the organochlorine pesticide residues were identified in samples collected from seven sites. Out of the major harvesting time sample from Channa and minor harvesting time from Limmu 69.29% and 17.81% were contaminated with DDT. A total 9.09% of the honey samples showed concentrations below the MRLs, 54.35 % of the honey samples exceeding the MRLs and 36.36% the samples were free from measurable pesticide residues. Comprehensive research into the effects of pesticides on honeybees and their products decline to which this study targeted to contribute is important.

ACKNOWLEDGEMENTS

First of all I would like to thank my lord Allah who helped me throughout my life.

I am very thankful to my advisors Dr. Argew Ambelu and Asgdom Malu for their help in designing this thesis report by guiding me thoroughly and giving me unreserved advice and also for their moral as well as technical supports and encouragement from start of this work up to end.

With heartfelt gratitude I wish to acknowledge my co-advisors Dr. Seblework Mokenen and Dr. Abera Gure for their guidance, time, critical comments, suggestions and the encouragement they provided throughout the period of my studies.

I also wish to thank Jimma University, Institute of Health for giving me master degree chance and for the study leave.

I also appreciate all the help provided by the technical staff in the Department of Environmental Health Sciences and Technology and Department of Chemistry, for their assistance during the laboratory experiments.

I also thank my family, friends and colleagues for their time and moral support.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION AND ACRONYMS

DDT	DichloroDiphenylTrichloroethane
EPA	Environmental Protection Agency
GAP	Good Agricultural Practice
GC-ECD	Gas Chromatography-Electron Capture Detector
GC-NPD	Gas Chromatography-Nitrogen Phosphorous Detector
HPLC	High Performance Liquid Chromatography
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LLE	Liquid-Liquid Extractions
LOD	Limit of detection
LOQ	Limit of Quantization
MoA	Ministry of Agriculture
MRLs	Maximum Residue Limits
PAs	Peasant Associations
PSA	Primary-Secondary Amine
QuEChERS	Modified Quick, Easy, Cheap, Effective and Safe
SLE	Solid Supported Liquid-Liquid Extraction
SPE	Solid-Phase Extractions
USD	United State Dollar

DEFINITION OF TERMS

Apiary: Beekeeping site

Co'ops: well organized cooperative engaged in honey transaction.

Honeybee: *Api millfera*

Honey flora: type of the flora which provides honeybees with high amount of nectar, pollen and honey-dew.

Limit of Detection (LOD):- The minimum amount or concentration of analyte that can be reliably distinguished from zero.

Limit of Quantification (LOQ):- The minimum amount or concentration of analyte in the test sample that can be quantified with acceptable precision.

Major harvesting:-the season: which comes after the known rainy season where large number of flowering times the flora from April to June.

Minor harvesting time: the season which comes from known rainy season where the honey quantity is low

Modern beekeepers: those who use modern bee hive for bee keeping.

Pesticide residue: - any specified substance in food, agricultural commodities, or animal feed resulting from the use of pesticide. It includes any derivatives of a pesticide, such as conversion products, metabolites, reaction products and impurities considered to be one of toxicological significance

Poly flora:- a large numbers of mixed flora or multi flora.

Recovery: The proportion of analyte (incurred or added) remaining at the point of the final determination from the analytical portion of the sample measured.

Residue: - Level of the pesticide in or on foods

Standard: - A substance of known identity and purity and/or concentration.

Traditional bee keepers: who are involved in traditional bee hive and hang the hives on the big flora in the forest.

Union: farmer's organization which involve honey transaction having with good alignment with bee keepers

1. INTRODUCTION

1.1 Background

Ethiopia have about seven million honeybee populations, and it's annual honey and beeswax production is estimated to be over 54,000 and 5000 tons, respectively ((MoA, 2013)). With this, the country is ranking ninth highest honey producer in the world and the leading producer of honey and beeswax in Africa. Beekeeping is significantly contributing to the beekeeper's livelihood and to the country's economy. To this fact, about 1.5 to 1.8 million households earn various levels of income a year from beekeeping. *Tej (*Honey wein) to which the major proportion of local honey goes is with high calorie supplements to traditional diets providing significant additional rural employment and incomes. Although not quantified for local conditions, beekeeping through pollination is highly contributing to crop yield, quality of environment and biodiversity conservation. The experience in the United States indicates that the value of pollination services provided by honeybees is estimated at 14.6 billion dollars annually (Begna D. et al, 2015)

The recent sudden decline of honey bee colonies is of global concern not only because of pollination services they provide in food production process, but also due to honey production among other benefits. While there are multiple variables, including poor nutrition, pests, diseases, and loss of natural bee habitat, negatively affecting bee health, it is becoming increasingly clear that the widespread use of pesticides on agricultural crops is a major factor. As such, to preserve honey bee health which is inextricably integrated with human health and to preserve the quality of bee by-products especially honey requires regular monitoring using rigorous analytical methods to confirm product quality (Irungu et al., 2016).

Honey is composed of over 300 compounds, mostly carbohydrates (>75 %) and water (~18 %), with minor components comprising of proteins, amino acids, vitamins, antioxidants, minerals, essential oils, sterols, pigments, phospholipids, and organic acids. Whereas these diverse ranges of compounds make it a nutrient rich food commodity, they also make it a highly complex analytical matrix especially when analysing the presence of trace compounds such as toxins, pesticide residues and other environmental pollutants. The presence of pesticide residues and other contaminants in honey can have adverse health effects on bees and humans decrease the quality of honey and devalue its beneficial properties (Bogdanov S, (2006).)

Nowadays, bee products are produced in an environment contaminated by various pollutants.

Pesticide application in crops can contaminate soil, air, water, and the flowers from which bees collect nectar for honey production, which may cause the introduction of those toxic chemicals into the food chain, affecting human health. In other words, hives could be contaminated by direct or indirect exposure. In the first case, the pesticide residues may originate from the treatment of bee hives with acaricides in the control of *Varroa destructor*. In the second case, the bees can get in touch with those pesticides during the foraging activities in an average radius of 3-6 km around the hive. The honey benefits can be suppressed by pesticides introduced to honey during its processing and arising from both agricultural and beekeeping practices (Eissa F. et al., 2014).

1.2 Statement of the Problems

Recently the global honeybees have presented a decline with considerable economic impacts and beekeepers. Abiotic stress from the lethal effects of pesticides is currently being scrutinized as a contributing factor to poorly understood bee colony losses. Pesticides are a class of chemicals or biological agent with properties designed to deter, kill, incapacitate, or otherwise limit damage by pests. The introduction of pesticide in Ethiopia to control agricultural pests' dates back to the 1960's. Although, the volume fluctuates across the pesticide types, the country on the average imports 3346.32 metric tons of pesticides annually. Using pesticides is widely spread following modern agriculture and areas with high crop framing parts of Ethiopia are yearly receiving different types and amounts of pesticides (Begna D. et al, 2015).

Typically, pesticide residues in honey occurs when bees in search for food, visit crops that have been treated with various agro-chemicals and/or when beekeepers use chemicals to control bee pests or diseases. So far, several researchers have reported various residues of pesticides in honey at varying concentrations confirming the need to constantly monitor the presence of pesticide residues in honey to assess any potential health risk and to ensure that its quality, whether as food or as a therapeutic, is not compromised. However, to date, only few studies have been carried out to monitor pesticide residues in honey produced from Africa (Irungu et al., 2016).

A recent study conducted in Kenya in 2010 detected four pesticides from beeswax and bee bread at very low concentrations (Muli et al., 2014). However, the cumulative levels and presence of pesticides in hive products over time can pose health problems for both honeybees and humans. Therefore there is the need to develop highly sensitive and selective analytical techniques that have the ability to analyze multiple pesticides simultaneously in hive products. Since honey is a complex analytical matrix, it is often necessary to clean-up the sample prior to instrumental analysis. This facilitates removal of matrix co-extractives that could result in enhancement or suppression of the signal of the targeted analytes during analysis. Conversely, this clean-up step is usually the most expensive, time consuming and laborious sample preparation step with the highest probability of introducing errors on recovery and method repeatability (Ferrer et al., 2011).

Conventional extraction/clean-up methods such as liquid-liquid (LLE) or solid-phase extractions (SPE), require large volumes of organic solvents and usually target pesticides from a single chemical class. Recently, extensive research has been geared towards finding more economical and environmental friendly methods that can yield good recoveries for a diverse range of pesticides. For instance, a recent

study compared four different methods for extracting 12 organophosphates and carbamates from honey and concluded that the choice of the method depends on the targeted analytes. In another example, two methods; solid supported liquid-liquid extraction (SLE) and a modified Quick, Easy, Cheap, Effective and Safe (QuEChERS) method for multiresidue analysis were compared using extraction efficiencies for determination of 30 LC amenable pesticides in honey at their MRLs. These authors concluded that in terms of recovery (ranged from 34 to 96 %) the methods had no significant difference but in terms of costs and time, the modified QuEChERS was better (Kujawski et al., 2014).

Honey bees readily fly up to 4 km in all directions from their apiary and thus have access to an area of about 50 km^2. They are such a best small sampler that can be used in geochemical exploration. The bee honey has been used as monitors of a variety of environmental contaminants, including heavy metals, low level radioactivity and pesticides (Bogdanov, 2007).

Honeybees are the main pollinating agents for numerous plants and fruit trees and hence, play a key role in agriculture and more generally in the maintenance of ecological biodiversity. They are the most affected farm animals by pesticides. Persistent pesticide use in agriculture can theoretically contaminate bee products Honeybees may be poisoned when they feed on nectar or pollen contaminated by pesticides. Bees may also be poisoned when they fly through a cloud of pesticide dust or spray or walk on treated parts of plant. Sometimes, colonies in the hives can be directly affected, but most commonly only field bees are killed or have their physiological functions altered (Sandra, 2007).

Previous data on honey production in Africa indicates that Ethiopia is the largest producer with an estimate of 41,233 tons of honey followed by Tanzania at 28,678 tons and Kenya at 25,000 tons in 2004- 2006. Recently, there is growing consensus that pesticides have killed honeybees and their food source plants and resulted in bee death and their products declines. However, the available information on the side effects of pesticides under local situations are little and incomplete as well as remaining obscure (Irungu et al., 2016).

Therefore, the main purpose of this study was analysis of organochlorine pesticide residues in honey collected from, different geographical sites in southwest Ethiopia this region often called the honey belt of Ethiopia, and known for its large honey production. It has a perennially green natural forest and high flora. As a result, bees in the area benefit from available flora and do not depend on beekeepers for

foraging. Consequently, the region records high levels of production with two to three harvesting seasons.

1.3 Motivation

I was working in an area which was very rich honey bee colony and peoples especially farmers and they fill the large number of traditional beehives in a very simple ways by by fumigating the beehives by using locally available leaves of flora which loved by honeybee known as Sombo and Baya and the like. But in a very short time within this potentially huge and rich area in honey bee colony shockingly lose its potential especially after extensive use of DDT for eradication of malaria. Even for agricultural crops including 2, 4 D and other pesticides. In the same way in my visit of my unclean in Limmu Shaye, who generate his income from beekeeping including his neighbours with the same work, I heard many complain of losing honeybee colony and its products (Honey).

Also the death of many colonies withdrawing the hives and in the same way here in Jimma many of the beekeepers in the Kersa Wereda have mentioned the same complains stated before especially the decline of bee colony and products. And they mentioned the use of DDT on khat production and for eradication of malaria. This indicated that there is possibility of having pesticides in honey products due to the honeybees collects pollen and nectars of those crops and different honey floras of the area which directly or indirectly exposed to different pesticide for production of honey. And decline of the bees colony may be due to the pesticides toxicity. Therefore, from this ground, I decided to investigate "Analysis of pesticide residue level in honey.

2. LITERATURE REVIEW

2.1 Pesticides

Pesticides are a central concept in the area of food safety. They are substances or combination of substances aiming to avoid, moderate or eliminate any pest. The definition of pest also includes insects, fungi, weeds, different animals and prions (United States Environmental Protection Agency, (2010).)

To state the amount of pesticide residues allowed in a food. "Maximum Residue Limit.", MRL, is used. MRL is often given in mg/kg and is determined by field trials combined with toxicological risk evaluations. The field trials are performed according to. "Good Agricultural Practice.", GAP, which supply guidelines for the trials as well as the assessment of the results. The minimum level of pesticide residues that can be determined by analysis is called Limit of Quantitation (LOQ). It is applied as threshold limit value when basic data from field trials is missing, if the pesticide was not intended for the specific food as well as if residue levels did not exceed LOQ during field trials (Alehagen, 2011)

2.2 Why pesticides are unique among environmental contaminants

Pesticides released into the environment may have several adverse ecological effects ranging from long-term effects to short-lived changes in the normal functioning of an ecosystem. Despite the good results of using pesticides in agriculture and public health, their use is usually accompanied with deleterious environmental and public health effects. Pesticides hold a unique position among environmental contaminants due to their high biological toxicity (acute and chronic). Pesticides by definition are toxic chemical agents. A pesticide is usually capable of harming all forms of life other than the targeted pest species. On account of this behavior then, they can best be described as biocides (capable of killing all forms of life). Although some pesticides are described to be selective in their mode of action, their range of selectivity is only limited to the test animals (Zacharia, 2011).

2.3 Pesticide residues in Honey

Honey is made of plant nectar, plant secretion or secretion by insects feeding on plants. Various compounds are ingested and then transformed to honey by *Apis mellifera* bees, commonly known as honeybees. Storing of this energy dense product in the hive is essential for feed and heating during the colder months of the year. According to the injunction of honey by the National Food Administration, honey is divided into three groups; depending on origin, depending on method of production/presenting

and bakery honey. There are several subgroups for methods of production and presenting, such as honey in honeycombs and filtered honey. Bakery honey may have undergone fermentation, been overheated or may hold a different taste, which makes it suitable for use in industrial baking as well as ingredient in the manufacturing of other foods (Alehagen, 2011).

The exact content of honey varies since nectar is collected from different sources, but the composition may look like presented in Table 1 (Mattson C O, (2009)). The sugar contains often of most fructose; approximately 40% whereas the glucose content is approximately 30%. But there are large variations; honey from rapeseed contains 55% glucose but the content is 11% in honey from heather. The amount of glucose determines how the crystallization proceeds. High percentages of glucose quicken the crystallization, especially if the water content at the same time is low. The crystallization is often avoided if the glucose percentage is lower than 25%. Temperature also affects the forming of crystals in honey, where the forming happens most quickly at 14ºC. A variation of enzymes plays an important role in the transformation from nectar to honey. Invertase is used when sucrose is split into fructose and glucose. Starch is decomposed with help of diastase and glucose oxidase forms gluconic acid and hydrogen peroxide from glucose, oxygen and water. The most common minerals found in honey are different kinds of potassium salts. The vitamin content is not very significant, with different vitamin B.'s being the most common. The different compositions of honey affect taste, color and texture which explain the wide variety of diverse types of honey (Mattson C O, (2009)).

Table 1. Components of honey and example of their different contents

Component	Amount (%)	Contents
Sugar	79	Fructose Glucose Sucrose Other sugars
Water	18	
Other components	3	Minerals Enzymes Trace elements Aromatic compounds Vitamins Acids

Studies show several beneficial physiological effects connected to intake of honey. Two studies showed positive effect on nocturnal coughing among children with decreased frequency, decreased severity and better quality of sleep for the child as well as the parent (Alehagen, 2011). A Cochrane study of 19 trials, including 2554 people, investigated if honey decreased healing time in acute and chronic wounds. The conclusion of the Cochrane study showed honey to an important aspect of honey consumption is the issue of food safety. There are three health hazards referred to intake of honey; infant botulism, toxic honey and pesticides. Infant botulism is caused by a toxin produced by the bacteria *Clostridium botulinum*. The toxin affects breathing, when blockage occurs of the neural impulse to the striated muscles .The environment of the gastric system and the intestines are different in infants compared to adults, which explains why bacteria can grow and produce toxins in infants. Whether honey really is the cause of infant botulism has been discussed and according to one source honey is only responsible for 5% of cases of infant botulism (Alehagen, 2011).

Toxic honey is produced when nectar is collected from certain flowers containing toxins, for example various species of rhododendrons and laurels. The plants produce grayanotoxins, which may cause symptoms as nausea, dizziness, low blood pressure and vomiting (U.S. Food and Drug Administration, (2010).). Another example is tutu (*Coriaria arborea)* bushes, where honey bees collect toxic honeydew from the sap sucking vine hopper. Poisoning can cause vomiting, dizziness, coma or even death (New Zealand Food SafetyAuthority, (2010).)

Pesticides are transferred to the honey by the bees as they pollinate different plants, where pesticides have been applied. Beekeeping also contributes to accumulation of pesticides in honey, as it often includes application of various substances inside the hive to prevent and eliminate common vermin (Bogdanov S, (2006).)

Previous studies of pesticide residues in honey show various results with most of them reporting findings to be low in regard to MRL. The number of analyzed samples in seven studies ranged from 24 to 111, with detection of pesticide residues between 25%-100% of the samples. One study performed in Spain found no pesticides in the samples. In a Turkish study the majority of the 109 samples contained pesticide levels exceeding MRL. However there is no conformity in the evaluation of results between the authors (Alehagen, 2011).

A study done on Determination of pesticide residues in honey: a preliminary study from two of Africa's largest honey producers Using liquid chromatography tandem mass spectrometry (LC-MS/MS), various clean-up methods were evaluated for efficient determination of multiclass pesticide contaminants in honey showed that the most efficient method was primary-secondary amine (PSA) sorbent which was significantly different from the others ($P < 0.05$; average recovery ~94 %) and was applied to analyze 96 pesticide residues in 28 retail honey samples from Kenya and Ethiopia. From our preliminary data, a total of 17 pesticide residues were detected at ~10-fold below maximum residue limit (MRL) established for food products except for malathion which was detected at almost 2-fold above its acceptable MRL. A highly efficient approach for determining pesticide residues in honey with good recoveries was developed. All residue contaminants were detected at levels well below their acceptable MRLs except malathion suggesting that the retail honey analyzed is safe for human consumption. Although PSA clean-up method was selected as the most efficient for cleaning honey samples, omitting the clean-up step was the most economical approach with potential applicability in the food industry (Irungu et al., 2016).

2.4 Farmers' perception and pesticide toxicity

A recent survey conducted in Adami Tullu district to assess farmers' perception of insecticide side-effects on honeybees tha was conducted in six peasant associations (PAs) and ten farmers were interviewed from each selected PA reported that On one hand, the results revealed that almost all (96.7%) of onion producers were aware of the undesirable -effects of insecticides on honeybees. On the other hand, the majority (96.7%) of the interviewed farmers applied insecticides (profenofos, endosulfan, diazinon, malathion, lambda-cyhalothrin, delteramethrin, dimethoate and DDT) at any stage of onion development whenever incidence of insect pests was noticed. The insecticide DDT has been banned from use in agriculture. It was found that 48.3% of the beekeepers abandoned beekeeping and they indicated that pesticide application was the major driving force for abandoning beekeeping and bee colony losses. About 53.3% of the interviewed farmers knew about the importance of honeybees in pollinating onion flowers. However, farmers in the study area did not pay due attentions to honeybees and to honeybees' role in onion seed production. As a result, farmers were spraying their onions at any developmental stages, including flowering. This eventually leads to loss of honeybee colonies and abandoning beekeeping in the area and reduction of onion seed yield (Melisie et al., March, 2016).

Another field assessment conducted on pesticides use and its economic impacts on the apiculture subsector in three districts of Amhara region (Dangila, Guangua and Mecha) in April 2014 by using Random household survey that conducted on a total of 270 respondents (90 per district) of which 137 were beekeepers and 133 none beekeepers. From the total respondents, 147 uses pesticides and Dimethoate 40% EC, Ethiolathion 50%, Karate 5EC, 2,4-D were the most often used pesticides. March, June, July and September are identified as pesticides applications months. From the total pesticides users (147), 114 (78%) apply before flowering stage, 25 both before and during flowering stages and the rest applies whenever they feel pests and/ or weeds occur. Besides, more than 60% of the respondents know pesticides kill honeybees and results in dwindling and absconding. Three years trends analysis of honeybee colony number and honey yield indicated dramatic decline mainly attributed to indiscriminate applications of pesticides. This assessment revealed indiscriminate uses of pesticides caused fatalities on 22987 honeybee colonies and incurred economic loss amounting USD 819291.37. This study is appropriate and timely to develop and implement effective development and extension strategies to minimize and/or control the ill effects of accidentally using pesticides (Begna D. et al, 2015).

2.5 Environmental Effect of the Pesticides

Pesticides, in addition to their potential negative effects on human health, pose adverse effects also on the environment (water, soil and air contamination, toxic effects on non-target organisms). In particular, inappropriate use of pesticides has been linked with: (1) adverse effects on non-target organisms (e.g., reduction of beneficial species populations), (2) water contamination from mobile pesticides or from pesticide drift, (3) air pollution from volatile pesticides, (4) injury on non-target plants from herbicide drift, (5) injury to rotational crops from herbicide residues remained in the field, (6) crop injury due to high application rates, wrong application timing or unfavourable environmental conditions at and after pesticide application (Eleftherohorinos, 2008.).

Many of the adverse effects of pesticides on the environment depend on the interactions between the physicochemical properties (vapour pressure, stability, solubility, pKa) of the pesticide, soil adsorption and soil persistence, the soil factors (pH, organic components, inorganic surfaces, soil moisture, soil microflora, soil fauna), the plant species, and the climatic variation (Eleftherohorinos, 2008.). Also, the toxicity, the dosage applied, the weather conditions prevailing after the pesticide application, and how long the pesticide persists in the environment could account for its adverse effects on the environment. Soil factors and weather conditions have long been recognised as the most important factors that affect the fate of the pesticide in the environment and consequently the activity, selectivity, and adverse effects on the environment (Monaco, Weller, & Ashton, 2002). Unfortunately, since these factors vary from site to site and from year to year, the results from any field study on the fate and behaviour of the pesticide are specific for one particular location and season. Therefore, for the environmental risk assessment, the behaviour and the fate of a pesticide are initially assessed by the calculation of the predicted environmental concentration (PEC), which in the United States is referred to as estimated environmental concentration (EEC) (Matthews, 2006). These concentrations are calculated for soil, water, sediment, and air, and the validation is performed by comparison with the data obtained from the three levels of tests (needed for approval-registration purposes) to assess the pesticide toxicity on key non-target organisms (Table 2).

Also, the toxicity exposure ratio (TER) is also calculated to determine whether the risk to the organism is acceptable or not (Hornsby, Buttler, & Brown, 1993). TER is calculated from the LC50 or equivalent measure (LD50, NOEC = no observed effect concentration) of the susceptibility of an organism divided by the PEC relevant to the situation in which the organism is living. In general, a detailed higher tier risk assessment is needed when TER is below 100, whereas a chronic risk assessment is required in the case of TER < 10. If TER is less than 5, the Annex VI of the EU Directive 91/414 EEC requires that ‘no

authorisation shall be granted…unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact occurs after the use of the product under the proposed conditions of use'. In USA, the risk quotient (predicted exposure concentration to predicted no effect concentration) is the inverse of TER and that is calculated by dividing the PEC with the indicated toxic dose (Matthews, 2006).

Table 2. The three level tests to assess pesticide toxicity on non-target organisms (adapted from (Matthews, 2006)).

Species	Tier 1 Acute toxicity	Tier 2 Reproduction test	Tier 3 Field test
Birds (bobwhite quail or mallard ducks)	LD_{50} (8–14 days)		Fish life cycle study
Freshwater fish (rainbow trout or minnows)	LC_{50} (96 h)	Effects on spawning	
Aquatic invertebrate (Daphnia, shrimp)	LC_{50} (48 h)	Full life cycle	
Non-target invertebrate (honey bee)	LD_{50} (48 h)	Effects of residues on foliage	Pollination field test
Non-target invertebrate (earthworms)	LC_{50} (14 days)	Effects of residues on foliage	
Aquatic plants (algae)	LC_{50} (96 h)	Plant vigour	
Other beneficial species	LD_{50} (48 h)		

Although the agricultural soil is the primary recipient of pesticides, water bodies that are adjacent to agricultural areas are usually the ultimate recipient for pesticide residues (Pereira, Antunes, Castro, Marques, Gonçalves, & Gonçalves, 2009). This issue is the reason for European authorities to require data (before the pesticide commercialization in Europe) related with the risk of non-target terrestrial and aquatic organisms when addressing potential adverse effects of pesticides on the environment.

Moreover, the use of certain environmental risk indicators as alternatives to direct pesticide impact measurement linked to methodological difficulties (*i.e.*, impossibility of measurement due to complexity of the system) or due to practical reasons (*i.e.*, time and costs) has also been a reality (Bockstaller et al, 2009). These indicators have already been used by Reus *et al*. (Reus et al, 2002) and Bockstaller *et al*. to assess potential risks of pesticides for water contamination, soil organisms (mainly earthworms), bees, air emissions, bioaccumulation, and human health.

Calculation of the environmental indicators used in these two studies was based on the pesticide persistence in soil (half-life, DT50), mobility in soil (organic-carbon adsorption coefficient, Koc) and toxicity to water (lethal concentration for aquatic organisms, LC50) and soil organisms (NOEC). Regarding the contribution of the environmental indicators on pesticide selection, the study conducted

by Reus *et al*. to evaluate 15 individual pesticide applications by using eight indicators showed the following: (1) some of the 15 pesticide applications had a high ranking (higher impact on the environment) with all the indicators used, but their ranking differed considerably when the score for the environment was concerned as a whole; (2) the ranking based on the indicator 'kilograms of active ingredient' did not correlate with most of the rankings obtained by the other pesticide risk indicators; (3) the pesticide risk indicators used gave similar rankings of the 15 pesticide applications for the individual region surface water, groundwater, and soil contamination. For the latter, the scores for surface water contamination were largely determined by the pesticide toxicity to aquatic organisms, whereas the scores for groundwater contamination were largely determined by DT50 and Koc. However, an exception was recorded with two pesticides that were found toxic or mobile although they had been applied at extreme low rates. These results indicate that new indicators with greater reliability than those already existing are needed to predict potential risk of pesticides and thus contribute to reduction of the adverse effects of pesticides on the environment (Reus et al, 2002).

2.6 Effect of Pesticides on honeybees

Agricultural pesticides for specific purpose are utilized to alleviate the problem of pests. Unfortunately, the honeybee is susceptible to many of these pesticides as the result of which the beekeeping industry is having an increasingly difficult time in maintaining adequate honeybee colonies in intensively cultivated areas, thereby affecting the pollination of cultivated crops. Pesticides could poison honeybees either through contact, direct spray, fumigation and feeding the contaminated forage (John, 2006).

2.7 Ecological importance of the honey bee

Wind is the main pollinating agent. In fact, most of the forest trees, almost all grasses and grains, with the exception of some that are completely self pollinated, and many weeds are wind-pollinated. The flowers of most wind-pollinated plants are either male or female. The male flowers produce an abundance of pollen to be transported by the wind. The female flowers usually have large stigmatic areas to receive the pollen (James Devillers and Minh-Hà Pham-Delègue, 2003).

Nearly 200 000 animal species play roles in pollinating the 250 000 species of wild flowering plants on our planet. Among them, about 1500 species of vertebrates such as birds (e.g. hummingbirds) and mammals (e.g. bats, lemurs) serve as pollinators. However, the main pollinators are insects: they include bees, wasps, moths, butterflies, beetles and so on. Bees are the most efficient and the only

dependable pollinators, because they visit flowers methodically to collect nectar and pollen and do not destroy the flower or the plant in the process (James Devillers and Minh-Hà Pham-Delègue, 2003).

Consequently, bees provide substantial benefits to the maintenance of the biodiversity and the productivity of both natural and agricultural ecosystems. However, with regard to agricultural ecosystems, it is important to stress that only 15 percent of the 100 or so crops that feed the world are serviced by domestic honey bees, while at least 80 percent are pollinated by wild bees and other wildlife (James Devillers and Minh-Hà Pham-Delègue, 2003).

Unfortunately, both wild bees and domestic honey bees are in decline. Thus, for example, the number of commercial US bee colonies plummeted from 5.9 million in the late 1940s to 4.3 million in 1985, and 2.7 million in 1995. The loss of one quarter of all managed honey bee colonies since 1990 signals one of the most severe declines US agriculture has ever experienced in such a short period. There are fewer bee hives in the US today than at any time in the past 50 years. This demise has been brought about by the spread of diseases and parasitic mites, invasion of Africanized honey bees, climatic fluctuations, industrialization, and exposure to pesticides and other chemicals. Xenobiotics can either poison the bees or impair their reproduction. These chemicals can also eliminate nectar sources for pollinators and/or deplete nesting materials. Consequently, there is a need to protect the honey bees and the others pollinators because of their ecological importance (James Devillers and Minh-Hà Pham-Delègue, 2003).

2.8 Significance of the study

This study will be expected to have some advantage that,

- Creating awareness among farmers about the role of honeybees in seed production is necessary;
- Enhance beekeepers and farmers on how to protect their bees from pesticide attack
- This particular study is considered to be important in providing original information for concerned bodies as well as government officials, who are responsible in protecting the honeybees from the harm of pesticides which are hazardous to human beings and the environment.
- This study will provide some insights in the safety of honey from Ethiopia and some baseline information for future studies on other components of the hive matrix in relation to honey bee colony losses.

3. OBJECTIVES

3.1. General Objective

- To evaluate of organochlorine pesticide residues in honey collected from selected sites in south western Ethiopia

3.2. Specific Objectives

- To determine the amount of pesticide residue in commercial available honey and harvested honey
- To analyze the type of pesticide residue present in commercially available honey and harvested honey
- To evaluate the result using international maximum residue limits (MRLs)

4. METHODOLOGY

4.1 Study area and period

The study was conducted in selected zones of South-western Ethiopia. This part of Ethiopia has relatively strong, well established cooperatives (co'ops) and unions for honey, compared to other regions. This can be found predominantly in Keffa, Bench Maji, Sheka and Jimma zone were bulk of Ethiopian honey comes from. Also these zones were selected based on geo-ecological characteristics, availability of considerably higher honey flora, consideration as honey belt of Ethiopia, high quantity and quality production of honey and market dominancy both domestically and for export purpose (Shenkute et al., 2012). Then the sample sites were selected based on the selection criteria prepared by principal investigator.

The study period was depends on the harvesting time of honey. South west and south eastern parts of the country, the major honey flow period occurs during May-June from September to November and April to May, after the two known rainy season's one bee population. In most parts of Ethiopian, beekeepers harvest honey once or twice a year depending on whether the area receives rain once or twice a year and depending on the flowering season of the honeybee flora. As such, the bulk of honey produced is poly-floral honey (honey sourced from different types of nectars) (McGill, 2016). Mostly this area is known for having two harvesting seasons. Major harvesting time is from April to June, and the minor is from November to January (Shenkute et al., 2012). As the result, the samples were collected in January 2017 (minor harvesting) and in April 2017 (major harvesting).

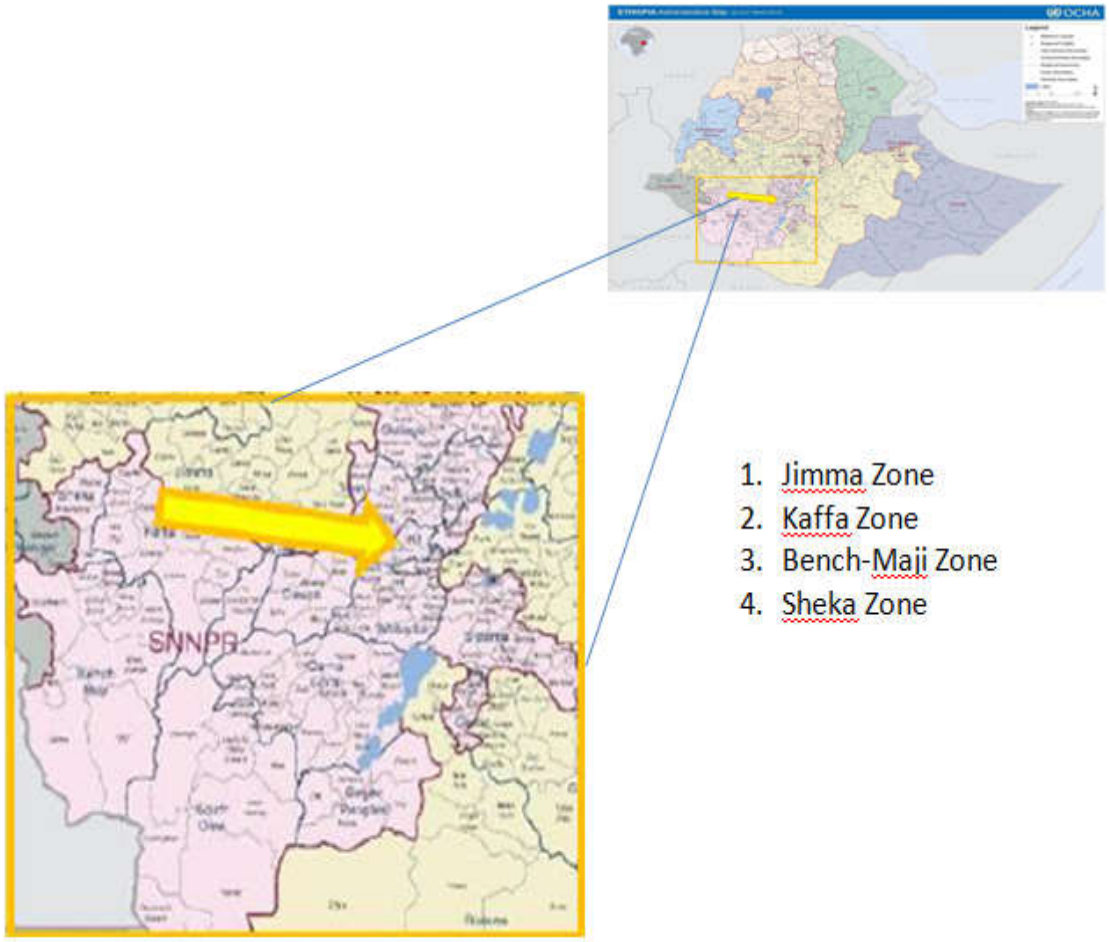


Figure 1 Map of study area indicating where the samples were collected.

4.2 Study design

An Experiments study was conducted to determine the pesticide residues level in honey. The samples were collected from the selected sites and transported Jimma University, Chemistry Laboratory for extraction. After extraction, the pesticide residues were analyzed by using Gas Chromatography with ECD.

4.3 Sample sites selection criteria

1) Those sites that have massive honey flora
2) Areas that have known in producing high quantity and quality of honey
3) Sites that have great market share and dominancy
4) Sites known by indigenous and cultural beekeepers
5) Those areas that are suspected to use pesticides in the production of agricultural crops.

Based on the above criteria, Gesha, Cena, Gimbo, Sheko, Masha, Limmu, Kersa, Gera, Sigmo, Manna and Gomma were selected.

This study was conducted in Kaffa, Sheka and Bench-Maji Zones of southwest Ethiopia. Out of the 21 districts that are found in the three Zones, the study was carried out in 5 districts, which were purposely selected based on the data of their honey production potential obtained from each Zone Agricultural Departments and proportion of districts in each Zone. Accordingly, Gimbo, Gesha and Chena from Kaffa Zone, Masha from Sheka Zone and Sheko from Bench-Maji Zone were selected. In these three zones, traditional beekeeping system is practiced by more than 99% of beekeepers (Shenkute et al., 2012).

4.4 Sample size and sampling techniques

A total of 22 samples were collected from the selected sites by using purposive sampling techniques. The samples was taken based on the two harvesting time of the honey. Which means one sample was taken from each site during the minor harvesting time and also one sample was taken from each site during major harvesting time.

All the samples from were collected from the co'ops (like Angacha), union (like Zambaba) Beekeepers association and known distributors available in the areas. The coops and union were selected due to

- they are well organized and the beekeepers are taken better care for transaction,
- they have the possibility of getting large number of beekeepers due to their fair purchasing price,
- they have long term consumer relation and traceability,
- they work with accountability
- they serve space for beekeepers
- they provide space for honey collection and proper storage

4.5 Honey Sample Collection

Honey samples were collected from selected 11 sites located in Jimma Zone, Sheka zone, Benji-Maji zone and Keffa zone. All the honey samples were collected from the known different co'ops, union Beekeepers association and known distributors. All the honey samples weighing 500 g for each sample was collected from each site. The collected samples were stored at room temperature in a dark place until extraction and analysis.

4.6 Chemicals and Reagents

Pesticides standards were obtained from Jimma University, Chemistry Department. All the pesticides standard were produced by PIPARK Scientific Limited, Northampton, UK and have Analytical Standard grade (97.9%). All organic solvents and reagents of analytical grade like Chloroform, Acetone, and Methanol were purchased from different suppliers and distributors.

4.7 Preparation Pesticide standards

10mg of the organochlorine pesticides standard was weighted into 10ml biker from each except Dibutyl Chloredate (20mg) and dissolved in 5ml methanol by using ultrasonication (Elmasonic). Then, the mixture emptied into a 10 ml volumetric flask to prepare 1000ppm stock solution. The prepared stock solution was stored in a deep freezer at -4 °C. For Dibutyl Chloredate, 20mg weighted into 10ml biker and dissolved in 5ml methanol by using ultrasonication (Elmasonic). Then, the mixture emptied into a 10 ml volumetric flask to prepare 2000ppm stock solution. From stock solution, Intermediate solution was prepared by taking 200µL from each stock solution except Dibutyl Chloredate (100 µL) into a 10 ml volumetric flask to prepare 20ppm Intermediate solution. A working standard was prepared by serial dilution of the Intermediate solution with n-hexane.

4.8 Sample preparation procedure using Dispersive Liquid–Liquid Microextraction (DLLM)

0.5 g of homogenized honey sample was dissolved in 3 mL of ultrapure water; and filtered using whiteman 42 filter paper; the resulting solution was spiked with surrogate standards (deuterized compounds) at 5 ng/g honey and mixed thoroughly. A mixture of 450 µL acetone (disperser solvent) and 100 µL chloroform (extractant) was prepared and rapidly injected into the sample to obtain an emulsion. After 20 s (including 5 s of shaking), the sample was centrifuged (5 min, 4.0 k RPM) and a two-phase solution was obtained. The resulting volume of sediment phase was 80 µL. During the extraction, a precipitate formed between chloroform and aqueous phase, which slightly impeded the collection of the small volume of chloroform. The chloroform phase at the bottom of the conical vial

was collected with a microlitre syringe, and 2 µL were injected on GC column. This is the optimized procedure, validated and applied in real sample analyses (Namies´nik:, 2012).

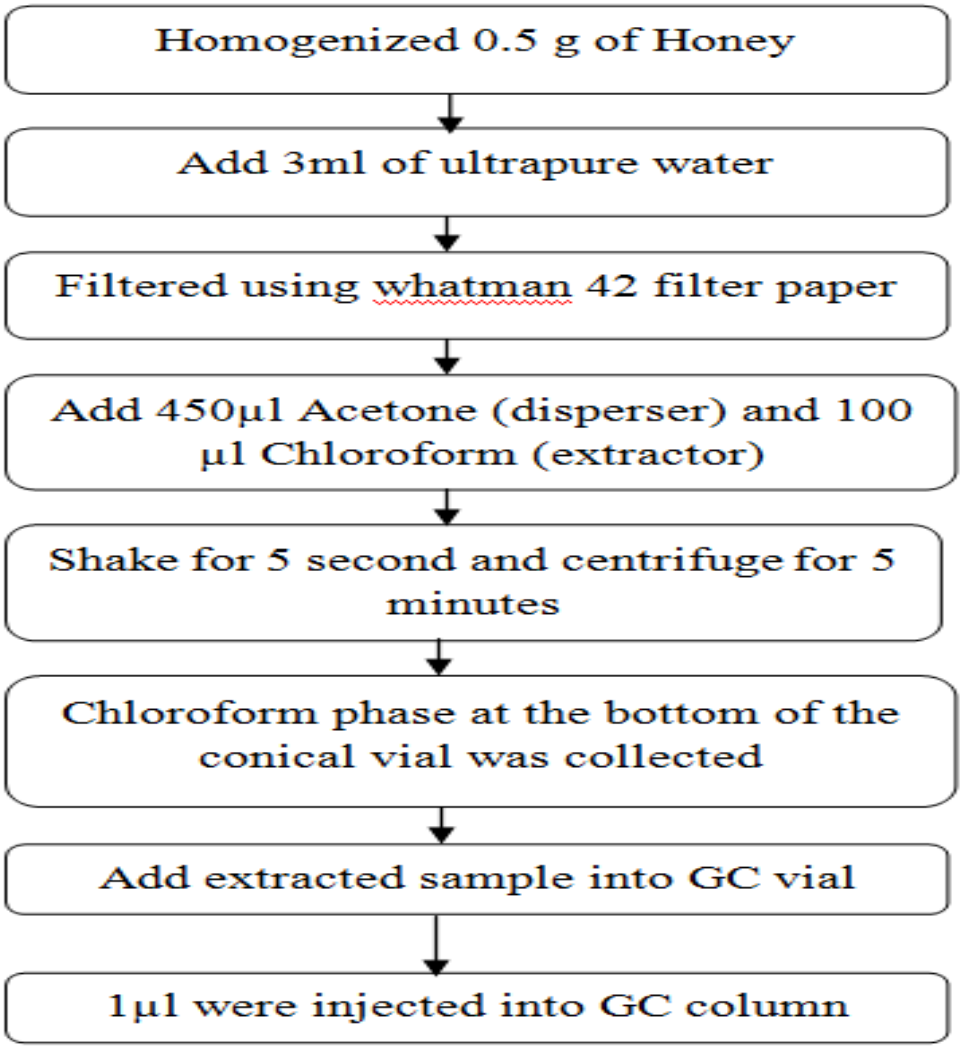


Figure 2: Sample extraction and cleanup procedure diagram

2.9 GC Conditions

All pesticide residues were determined by gas–liquid chromatography with an electron capture detector (GC-ECD; Agilent Technologies 7890 A) and an auto-sampler. An HP-5 capillary column (30mx0.25mm inner diameter; 0.25-µm 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°C , ramped at 30°C min^{-1} to 180°C, ramped at 3°C min^{-1} to 205°C, held for 4 min, ramped at 20°C min^{-1} to 290°C, held for 8 min, ramped at 50°C min^{-1} to 325°C. The total GC run time was 27.92 min. Helium (99.9999% purity) was used as a carrier gas at a flow rate of 20mLmin^{-1} and nitrogen as makeup gas at a flow rate of 60mLmin^{-1}. An aliquot of 1 mL was injected in split mode at a split ratio of 10:1 and injection temperature of 280°C. The pesticide residues were detected with m-ECD operating at a temperature of 300°C. The pesticide residues in each honey sample were analyzed in triplicate, and the mean concentration was computed accordingly.

4.10 Quality assurance

Method validation was applied by running a number of pesticides standards of known concentration and spiked honey samples. The recovery values for interested pesticides ranged between 70% to120 percent.

The linearity studies performed with five samples of known concentration showed the linear relationship, with 'r' value between the calibration curves and concentration of pesticides. Coefficient variations for repeatability studies were below 5% strongly confirm the validity of this method. Limit of detection (LOD) and limit of quantification (LOQ), were determined by running at least five samples of known concentration for each pesticide.

4.11 Data processing and analysis

After the samples were injected into GC-ECD, the data was printed and taken for further analysis of the result. The data was exported in to the Microsoft Excel for the calculation of the pesticide residues using the calibration curve equation. After that the residues level detected were quantified and presented by using graph and tables.

4.11 Ethical Consideration

Ethical clearance was obtained from the Ethical Review Board of Jimma University, College of Health Sciences. Permission was obtained from each kebele administration office. Verbal informed consent was obtained from responsible bodies of the households prior to the sample collection. Confidentiality and privacy of the information was assured and maintained.

5. RESULTS

5.1. Validation Information

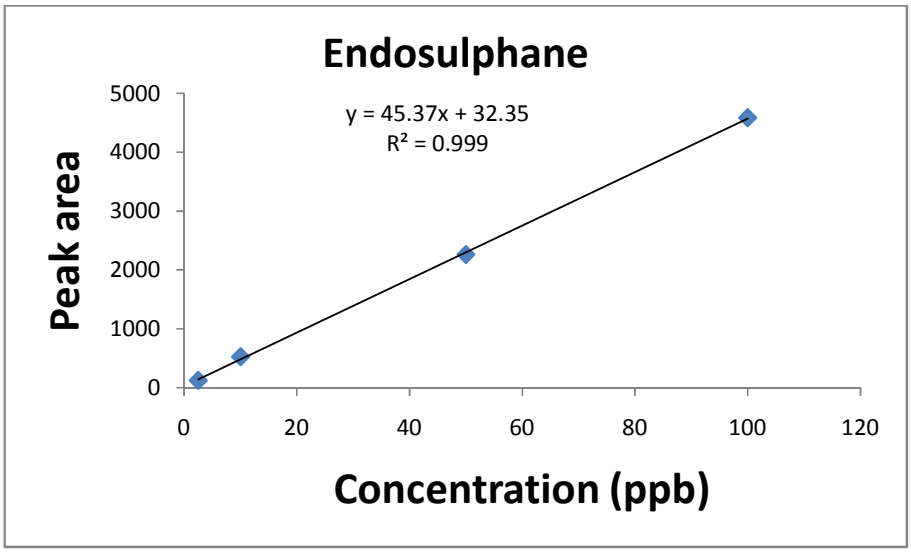
LOD and LOQ were determined as the lowest concentrations yielding a signal-to-noise (*S*/*N*) ratio of 3 and 10, respectively, where their values are 3S/N and 10S/N respectively.

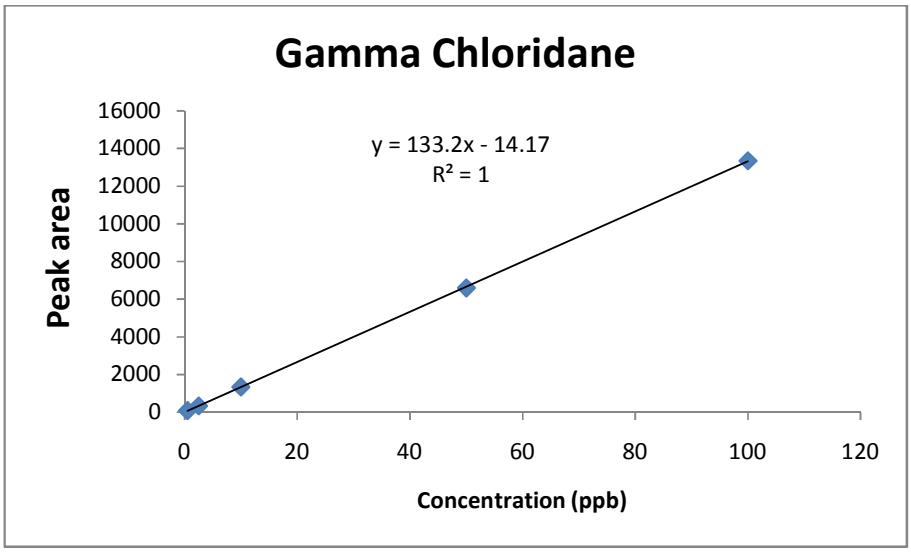
The percentage recoveries of the organochlorine pesticides were found to be acceptable ranging from 68.59% for Endrin to 113.41% for Dibuthylchlorepoxide , which indicates that the reproducibility of the method was satisfactory (Table 1). The limits of detection (**LOD)** and limits of quantification (**LOQ)** varied from 0.06-0.72 and 0.30-2.40, respectively.

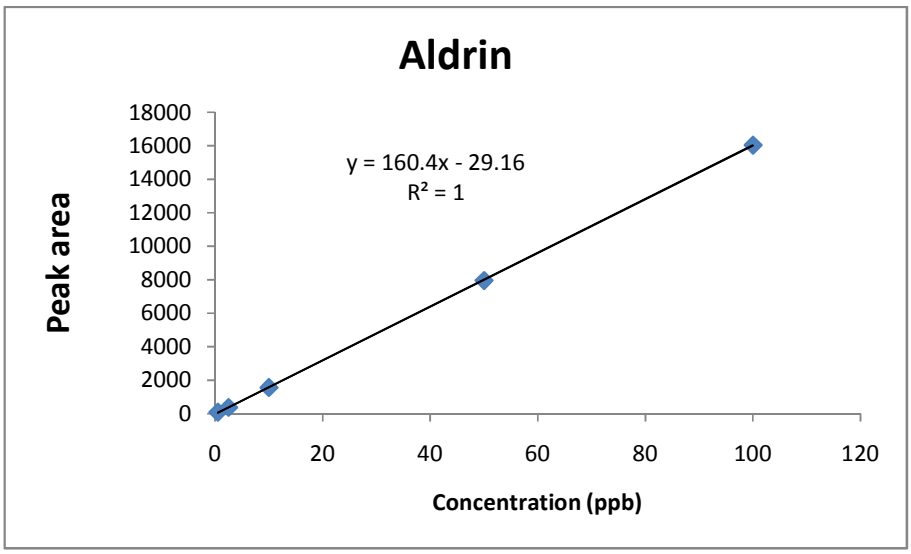
Table 1: The Percentage Recoveries and validation information of the studied pesticides

Pesticides standard	**Percentage Recovery**	**Limits of Detection (mg/kg)**	**Limits Quantification (mg/kg)**
Aldrin	97.73	0.10	0.34
Dibuthylchlorepoxide	113.41	0.16	0.54
γ-chloridane	103.62	0.72	2.40
p,p-DDT	112.56	0.14	0.48
Endrin	68.59	0.15	0.50
Endosulfan Sulfate	86.78	0.15	0.50
Dieldrin	78.45	0.06	0.30
Methoxychlor	98.23	0.15	0.50
Heptachlorepoxide	75.94	0.15	0.50

The calibration curves were obtained by preparing and injecting eight different concentrations of the pesticide standards with n-Hexane in a range of 0.05-100ng/ml. Linear spiked calibration curves for all the interest pesticides were obtained with correlation coefficient (r^2) >0.999. The calibration curves for some of pesticide standards were presented in **Figure 1**.


Endosulphane
y = 45.37x + 32.35
R² = 0.999
Peak area
5000
4000
3000
2000
1000
0
0
20
40
60
80
100
120
Concentration (ppb)


Gamma Chloridane
y = 133.2x - 14.17
R² = 1
Peak area
16000
14000
12000
10000
8000
6000
4000
2000
0
0
20
40
60
80
100
120
Concentration (ppb)


Aldrin
y = 160.4x - 29.16
R² = 1
Peak area
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
20
40
60
80
100
120
Concentration (ppb)

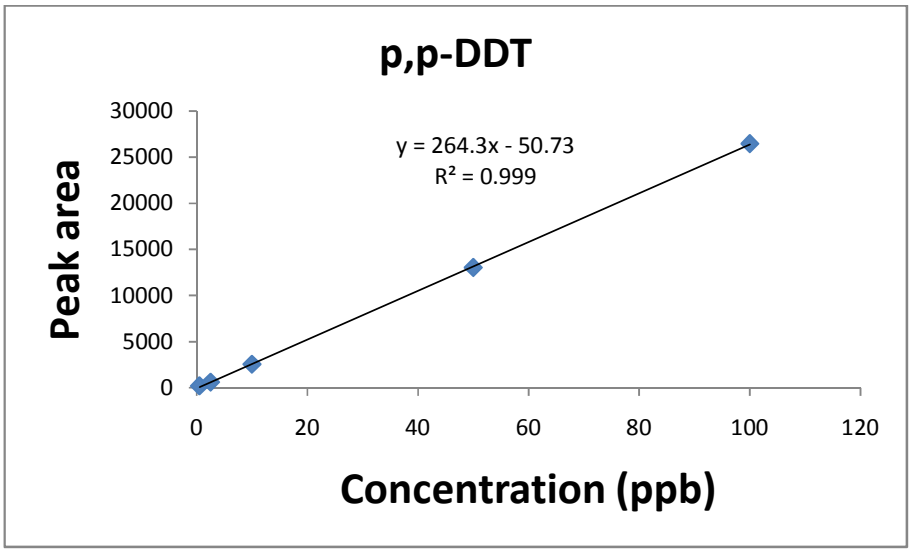


Figure 3 Calibration curves for different Organochlorine Pesticide standards

5.2 Pesticide residues in honey samples

A total 22 samples were collected from 11 site selected in four different zones of southwest Ethiopia and were investigated for the presence of nine organochlorine pesticide residues. The investigated pesticide compounds were: Aldrin, Dibuthylchlorepoxide, γ-Chlordane, p, p'-DDT, Dielderin, Endosulfan Sulfate, Endrin, Methoxychlor and Heptachlorepoxide. From each site two samples were taken based on the harvesting time of the honey which is minor harvesting time and major harvesting time.

Out of the total samples analyzed, the organochlorine pesticide residues were identified in samples collected from seven sites including Channa (from both major and minor harvesting time), Gesha (from minor harvesting time), Shabe (from both major and minor harvesting time), Sigmo (from minor harvesting time), Gera (from major harvesting time), Limmu (from both major and minor harvesting time) and Kersa (from minor harvesting time). The samples analyzed from Channa have high amount of organochlorine pesticide residues in respective of other samples obtained from different sites. There are seven organochlorine pesticide residues identified from Channa major harvesting time sample including Aldrin (0.0024mg/kg), DDT (0.564mg/kg), Endrin (0.0015mg/kg), Endosulfane sulphate (0.0035mg/kg), Dieldrin (0.00375mg/kg), Methoxychlor (0.0055mg/kg) and Heptachlorepoxide (0.028mg/kg) with the highest concentration of DDT and Heptachlorepoxide (Figure 4). All the organochlorine pesticide residues analyzed were identified in the sample obtained from Channa Minor

Harvesting time with the predominant concentration of Aldrin (0.01625mg/kg) and γ-Chlordane (0.07177mg/kg). Also the residue concentration in sample obtained from Limmu manor harvesting time were indicated that there are some residue identified in this site with the highest level of p,p-DDT (0.145mg/kg) and Dieldrin (0.0137 mg/kg).

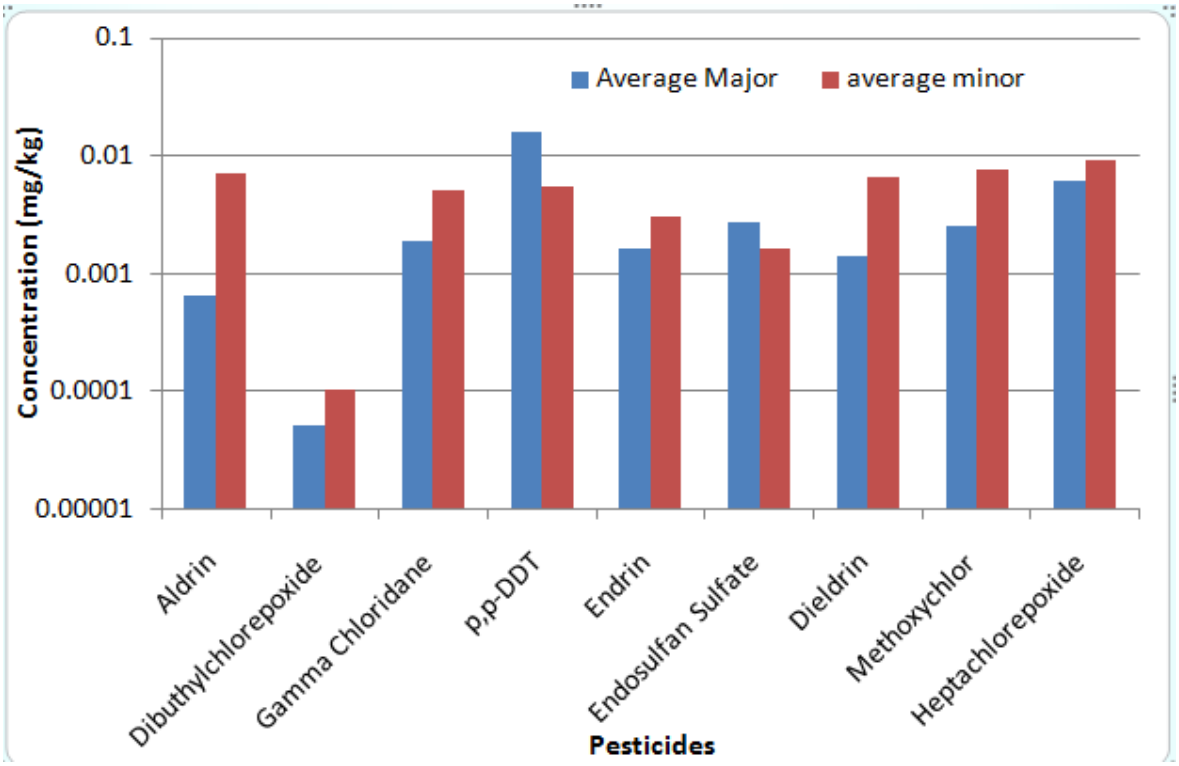


Figure 4: Average organochlorine pesticide residues identified in honey samples of Major Harvesting Time and Minor Harvesting Time in Southwest Ethiopia

From the total organochlorine pesticide residues identified, only two samples obtained from the Channa in Major and Limmu in minor harvesting time) were above the detection limit. Out of the major harvesting time sample from Channa, 69.29% were contaminated with p,p-DDT (Figure 1). Also the sample collected during minor harvesting time from Limmu has p,p-DDT residue with the percentage of more than half of the sample 17.81%. This is due to there is widespread and uncontrolled use of pesticide especially DDT. This practice include development of agricultural investment in the area lead the investors to get high benefit they are using pesticides intensively for agricultural purpose which contaminate the nectar of cultivated plant and the floras around in which honeybees use the contaminated pollen and nectar for production of honey. This action leads to the accumulation of Organochlorine pesticides to the bees wax due to lipophilicity nature of Organochlorine pesticides. Consequently the Organochlorine pesticides enter in to honey.

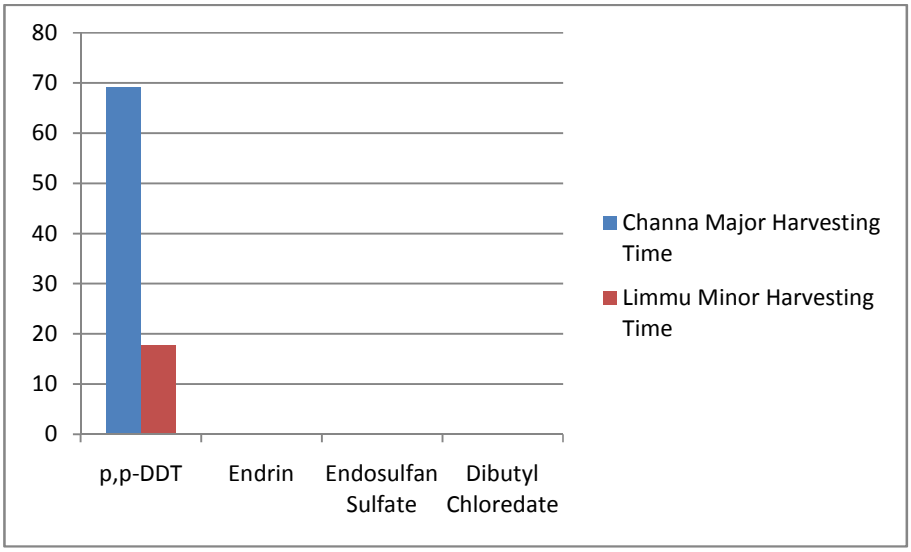


Figure 5: Percentage distribution of organochlorine pesticide residues detected in honey sample obtained from Channa and Limmu, Southwest Ethiopia.

Based on their harvesting period, the honey samples i major harvesting time have high concentration level of residue than the minor harvesting time. Especially Heptachlorepoxide was the predominant organochlorine pesticide residues identified with the average concentration of 0.006 mg/kg from Major Harvesting Time honey sample and followed by p.p-DDT with the residue concentration of 0.016 mg/kg. Dibuthylchlorepoxide was the lowest organochlorine pesticide residues identified in all samples collected from selected

sites and had residue concentration of 0.011 mg/kg (table 2). Also Heptachlorepoxide was the predominant organochlorine pesticide residues identified from Minor Harvesting Time with the residue concentration of 0.009mg/kg.

Table 2: Average organochlorine pesticide residues identified in honey samples of major harvesting time and minor harvesting time in southwest Ethiopia.

Pesticides	**Major Harvesting Time**	**Minor Harvesting Time**
	Average residue (mean±SD) in mg/kg	**Average residue (mean±SD) in mg/kg**
Aldrin	0.0007±0.00117	0.0072±0.0067
Dibuthylchlorepoxide	0.00005±0.00003	0.0001±0.0001
Gamma Chloridane	0.0019±0.0027	0.00523±0.014
p,p-DDT	0.0163±0.0269	0.0054±0.0789
Endrin	0.0017±0.0023	0.003±0.0027
Endosulfan Sulfate	0.0028±0.0027	0.0017±0.0021
Dieldrin	0.0014±0.0016	0.0065±0.0006
Methoxychlor	0.0025±0.0028	0.0079±0.0007
Heptachlorepoxide	0.0062±0.0021	0.0093±0.0004

The European maximum residue limits (MRLs) were followed due to lack of Codex MRLs of target pesticides on honey. The European regulation 396/2005 EC set the limit at 10 μg kg^{-1} for substances for which no MRL had been established. Since 1 September 2008 the European Commission has set new MRLs, which mostly are between 10 and 50 ng g^{-1} in honey. Comparisons of Average mean concentration of identified residues with OCP MRLs established by the European Union (EU) was summarized in Table 3. As indicated in the results the analyzed samples had residue of above the MRL for honey set by European Union (EU) for only p,p-DDT in major harvesting time. (European Union, 2005). Even though p,p-DDT above the MRL, it's difficult to decide whether the concentration is sufficient to cause consumer risk. So, further investigation of consumer risk assessment is important.

Table 3: Comparisons of Average mean concentration of OCP residues with European Union for honey

Pesticides	Major Harvesting Time (mg/kg)	Minor Harvesting Time (mg/kg)	EU MRLs (mg/kg)
Aldrin	0.001	0.007	0.01
Dibuthylchlorepoxide	0.0005	0.0001	0.01
Gamma Chloridane	0.002	0.005	0.01
p,p-DDT	0.016	0.005	0.01
Endrin	0.002	0.003	0.01
Endosulfan Sulfate	0.002	0.002	0.05
Dieldrin	0.001	0.007	0.01
Methoxychlor	0.002	0.008	0.01
Heptachlorepoxide	0.006	0.003	0.01

A total 9.09% of the honey samples collected in apiaries of the southwest under study, showed concentrations above the MRLs, 54.55% of the honey samples were below the MRLs and 36.36% the samples were free from measurable pesticide residues. Generally, the result showed that 90.91% of the honey samples are less than MRLs this is probability due to in the there is no much agricultural crops which mostly force to use agrochemicals. Most of the hives are very far from their small farming area as they practice traditional beekeeping systems.

The reason why 9.09% of the honey samples are may be due to huge government and individual large scale indigenous farmers which uses agrochemical for high production of their cultivated crops. Additionally due to high prevalence of malaria in the areas, the use of DDT is predominant in order to prevent and control malarial vector.

6. DISCUSSIONS

Results this study revealed that DDT was the most frequently detected pesticides in honey obtained from the study area. Although organochlorine pesticide usage has been completely prohibited by law, the results obtained could be expected, because those pesticides and their metabolites have been extensively used and are still present in the environment, owing to their high persistence. Organochlorine pesticides are lipophilic substances and consequently are soluble and stable in beeswax. Therefore, an amount of these substances gradually migrates from wax into the stored honey (Eissa F. et al., 2014).

Many studies have shown that residues of organochlorine pesticides (OCPs) bio-accumulate in plants from polluted soil from historical agricultural applications. Bio-accumulation levels in plant tissues can reach 10 to 1,000 times greater than those in ambient environmental media such as air and water. OCPs can enter the food chain via not only fatty products, but also non-fatty products such as honey (Eissa F. et al., 2014).

The result of the present study is in accordance with honey examinations in different geographic regions, which were performed during the year 2010. During this examination, it was determined that concentrations of DDT derivatives in developing countries was in the range from 0.41 to 3.54 ng g^{-1}, while the value of derivatives of DDT in developed countries ranged from 0.1 to 4.35 ng g^{-1}. The result of the present study for DDT is similar with this values which is 0.01627mg/kg and 0.005435 mg/kg for major harvesting time sample and minor harvesting time sample, respectively. The concentration of HCH isomer, primarily lindane, significantly differs in countries that are already developed, in compares to still developing countries. An average value of lindane content in developed countries ranged from 1.6 to 8.7 ng g^{-1}, while in developing countries it ranged from 0.21 to 4.78 ng g^{-1} (Wang J., 2010).

Compounds detected and the range of concentrations is comparable with other studies. Antonescu C. analyzed OCPs in 265 honey samples collected in Romania and found that 50% and 25% were positive for HCHs and dichlorodiphenyltrichloroethanes (DDTs), respectively (Antonescu C., 2001). But our result for the major harvesting time sample from Channa, 69.29% were contaminated with p,p-DDT which greater than the study in Romania that found 25% were positive for dichlorodiphenyltrichloroethanes (DDTs). This is due to

there is widespread and uncontrolled use of pesticide especially DDT in the study area. This practice include development of agricultural investment in the area lead the investors to get high benefit they are using pesticides intensively for agricultural purpose which contaminate the nectar of cultivated plant and the floras around in which honeybees use the contaminated pollen and nectar for production of honey. This action leads to the accumulation of Organochlorine pesticides to the bees wax due to lipophilicity nature of Organochlorine pesticides. Consequently the Organochlorine pesticides enter in to honey.

Wang et al. found that honey samples from developing countries generally contained higher concentrations of HCHs, ΣDDTs, Σchlordanes, and HCB than those from developed countries. This result is also similar with our value since DDT detected at high concentration. Malathion residues were detected in all the samples of locally produced honey, in Bauru (State of Sao Paulo, Brazil) during 2003-04, in a high concentration, owing to its applications to control dengue mosquitoes in the area studied (Rissato S.R., 2007). In Ethiopia, Malathion residues were detected in honey samples analyzed previously. This result leads to the possibility of presence of Malathion residues in our samples. But this pesticide left unstudied due to absence of standard and GC-MS machine.

A multi residue analysis was developed to quantify 80 environmental contaminants, pesticides and veterinary drugs belonging to different chemical classes, in honeys, honeybees, and pollens from France. In total, 36 compounds were detected but only 10 compounds were detected in all the matrices that can be used by beekeepers to combat varroa (Wiest L., 2011). Concentration levels of 30 pesticide residues were measured in honey samples collected from apiaries in northern Poland (Pomerania) using a method based on QuEChERS extraction followed by liquid chromatography- tandem mass spectrometry with electron spray ionization (LC-ESI-MS/MS). 29% of the samples were found positive for at least some of the target compounds, and profenofos was the most abundant pesticide (Barganska Z., 2013).

The result of this study revealed that DDT is one of the pesticides residues identified at above the EU MRLs which is also related with the study done by Kolonkaja et al. determined the 13 residues of organochlorine pesticides including α- and β- HCH, lindane, aldrin, dieldrin, endrin, DDT and its derivatives. It was concluded that although no longer used, all of these pesticides and their metabolites are still present in honey because of its persistence. Similarly,

in this study residues of endosulfan, aldrin and DDT were found in all samples (Kolonkaja D., 2001).

In this study different types organochlorine pesticides residues were identified including p,p-DDT, Heptachlorepoxide, and γ-Chloridane. Similar results were reported in the study Residues of Organochlorine Pesticides in Different Types of Honey in the Pannonian Region Republic of Serbia. It has been determined that in the examined samples of all detected pesticides (aldrine, endosulfan and sum of DDD), lindane is present at a significantly higher degree (concentration). During the examination, it was determined that the content of lindane was the highest in acacia honey, where an average value of measurement is 4.45 ng g^{-1}, while the DDT derivate sum, was on highest level in forest honey and it was 5.49 ng g^{-1}. (Kartalovic et.al, Residues of Organochlorine Pesticides in Different Types of Honey in the Pannonian Region Republic of Serbia, 2015)

A total 9.09% of the honey samples collected in apiaries of the southwest under study, showed concentrations above the MRLs, 54.55% of the honey samples were below the MRLs and 36.36% the samples were free from measurable pesticide residues. Generally, the result showed that 90.91% of the honey samples are less than MRLs this is probability due to in the there is no much agricultural crops which mostly force to use agrochemicals. Most of the hives are very far from their small farming area as they practice traditional beekeeping systems.

The reason why 9.09% of the honey samples are may be due to huge government and individual large scale indigenous farmers which uses agrochemical for high production of their cultivated crops. Additionally due to high prevalence of malaria in the areas, the use of DDT is predominant in order to prevent and control malarial vector.

Farms producing agricultural crops near to the hives and agricultural practices conducted in the study areas are similar to each others; this is given by the climatic conditions of these regions, which allow to the farmers planting the same crops. For this reason, the pesticides used around the hives are the same and only dose and frequencies of pesticide application vary.

This study indicates that in agricultural areas with developed apiculture, useful information about the occurrence and distribution of pesticide residues due to crop protection treatments can be derived from the analysis of randomly collected honey samples used as bioindicators. Because it is necessary to provide safe food to the consumers, it is essential that adequate monitoring should be in place to eliminate the possibility of the presence of the residues in food commodities in excess of the prescribed levels.

7. Conclusions and Recommendations

7.1 Conclusions

The data obtained in this study indicate that only out of the samples obtained from eleven sites, only the sample of seven sites were identified for organochlorine pesticide residues. A total 9.09% of the honey samples collected in apiaries of the southwest under study, showed concentrations above the MRLs, 54.55% of the honey samples were below the MRLs and 36.36% the samples were free from measurable pesticide residues. The pesticides exceeding MRLs were DDT. The data obtained from this study and the high frequency of detection of pesticide residues in honey samples are probably an indication of the widespread use of pesticides in the area of study.

In addition, this study revealed, for the first time that the bees and/ or hives in the study areas are exposed to chemical contaminants, including some insecticides such as organophosphorus and organochlorine pesticides, which represents a risk to bees.

7.2 Recommendations

Regulatory body that oversees the total supply, transportation, storage, appropriateness etc of pesticides at all levels should be in place.

The EPA Pollinator protection label standard should be followed by the producers.

Close follow up of pesticide use by the agricultural office of the areas where pesticide residues detected should be recommended.

Proper communication and training must be given for the beekeeping farmers on application time of pesticides.

Crops weed management practices known by the community like hand weeding should be capitalized at least for two reasons: to protect bees and the environment; and to ensure the products are natural.

Comprehensive research into the effects of pesticides on honeybees and their products decline to which this study targeted to contribute is important.

The use of Bio-pesticides is also recommended

Further investigation of human or consumers risk assessment should be undertaken in order to identify and solve the problem and toxicity associated with pesticides residues (DDT) identified and detected in this honey samples.

REFERENCES

Alehagen, M. (2011). Development of a method for determination of pesticide residues in honey using liquid chromatography tandem mass spectrometry.

Anon. (1993). *Pesticide Residues in Food. Rome:* . U.S. America: FAO.

Antonescu C., M. C. (2001). Environmental pollution and its effects on honey quality. Rom. Biotech. Lett., 6, 371.

Arthur, e. a. (2011,). Levels of Organochlorine Pesticide Residues in Grasscutter (Thryonomys swinderianus) Tissues. *Research Journal of Environmental and Earth Sciences,* , 3(4), 3(4), 350-357.

Atkins, E. L. (1973). *Toxicity of pesticides and other agricultural chemicals on honeybees.* 2287.: University of California Division of Agriculture and Social. Leaflet. .

Barganska Z., S. M. (2013). Pesticide residues levels in honey from apiaries located of Northern Poland. Food Control, 31, 196.

Begna D. et al, .. (2015). Assessment of Pesticides Use and its Economic Impact on the Apiculture Subsector in Selected Districts of Amhara Region, Ethiopia. *J Environ Anal Toxicol* , 5: 267.

Bhanti M, T. A. (2007,). Contamination of vegetables of different seasons with organophosphorous pesticides and related health risk assessment in northern india. *Chemosphere.,* , 69(1), 63-68.

Binelli, A. a. ((2004)). Risk for human health of some POPs due to fish from Lake Iseo. . *Ecotoxicology and Environmental Safety,* , 58; 139–145.

Bockstaller et al, .. (2009). Comparison of methods to assess the sustainability of agricultural systems. *A review. Agron. Sustain. Dev. , 29*, 223-235.

Bogdanov S. ((2006).). Contaminants of bee products. *Apidologie,* , 37:1-18.

Bogdanov, S. H. (2007). Minerals in honey: environmental, geographical and botanical aspects. Journal of Apicultural Research and Bee World. 46 (4): 269-275.

Codex Alimentarius, .. (2017). <http://www.codexalimentarius.org/standards/ pesticide-mrls/en/> (accessed August, 2017).

Eissa F. et al. (2014). Determining Pesticide Residues in Honey and their Potential Risk to Consumers. *Pol. J. Environ. Stud.* , Vol. 23, No. 5, 1573-1580.

Eleftherohorinos, I. (2008.). Weed Science: Weeds, Herbicides, Environment, and Methods for Weed Management; AgroTypos: Athens, Greece, .

European Union, .. (2005). European Parliament and Council, Regulation (EC) No. 396/2005 of the European Parliament and of the Council of 23 February 2005 on Maximum Residue Levels of Pesticides in Products of Plant and Animal Origin and Amending Council Directive 91/414/ECC.

FAO- Food and Agricultural. ((1983).). *Compilation of legal limits for hazardous substances in fish and fishery products.* U.S.A: FAO Fishery Circular,.

Ferrer et al. (2011). Overcoming matrix effects using the dilution approach in multiresidue methods for fruits and vegetables. *J Chromatogr A.* , 1218:7634.

Furst, P. F. (1990). Levels of PCDDs and PCDFs in food-stuffs from the Federal Republic of Germany. *Chemosphere,* , 20 ; 787–792.

George, W. (1978). *Pesticides Theory and Application.* U.S.America:: University of Arizona.

CTED FROM COTTON GROWING BELT OF PUNJAB, PAKISTAN. *Pak. J. Agri. Sci.,* , Vol. 51(2), 321-325 .

HBRC. (1997.). *(Holeta Bee Research Center)., Beekeeping Training Manual (unpublished),*. Ethiopia. 207 pp.

Hornsby, A., Buttler, T., & Brown, R. (1993). Water quality and the environment. Managing pesticides for crop production and water quality protection: Practical grower guides. *Agr. Ecosyst. Environ. , 46,*, 187-196.

Irungu et al. (2016). Determination of pesticide residues in honey: a preliminary study from two of Africa's largest honey producers. *International Journal of Food Contamination 3:14* , 3:14.

James Devillers and Minh-Hà Pham-Delègue, .. (2003). *Honey Bees: Estimating the Environmental Impact of Chemicals.* London and New York: Taylor & Francis e-Library.

John, R. (2006). In *Pesticides, a guide to their effects on honey bees,* (p. 19 pp.). Tamworth.: Intensive Industries Development,.

Kartalovic et.al, .. (2015). *Wulfenia Journal Klangenfurt Austria* , Vol 22 No. 2.

Kartalovic et.al, .. (2015). Residues of Organochlorine Pesticides in Different Types of Honey in the Pannonian Region Republic of Serbia. *Wulfenia Journal Klangenfurt Austria* , Vol 22 No. 2.

Kolonkaja D., K. O. (2001). Determination of organochlorine pesticide residues in honey Project report, TKV.

Kujawski et al. (2014). Determining pesticide contamination in honey by LC-ESI MS/MS – comparison of pesticide recoveries of two liquid-liquid extraction based approaches. *LWT Food Sci Technol.* , 56:517–23.

LeDoux, M. (2011). Analytical methods applied to the determination of pesticide residues in foods of animal origin. A review of the past two decades. . *J. Chromatogr. A.,* , 1218 (8):1021–1036.

Linda S. S., E. A. (2009). Exposure as part of a systems Approach for Assessing Risk. . *Environmental Health Perspectives,* , 117(8):1181-1184.

Mansour, S. (2004). *Pesticide exposure-Egyptian scene.* . 91-115: Toxicology198.

Maroni M., F. A. (2006). Risk assessment and management of occupational exposure to pesticides in agriculture. *La Medicina Del lavaro.,* , 97(2):430-437.

Matthews, G. (2006). Pesticides: Health, Safety and the Environment; Blackwell Publishing: Oxford, UK, .

((2009)). In L. J. Mattson C O, *Bin till nytta och nöje.* (4th Edition. ed.). Morgongåva. Hallvigs Tryckeri.

Melisie et al. (March, 2016). Farmers' insecticide use practice and its effect on honeybees (Apis mellifera) foraging on onion flower in Adami Tullu district of Ethiopia. *Global Journal of Pests, Diseases and Crop Protection* , Vol. 4 (1), pp. 139-145.

Miranda J. R., M. C. (2004). Muscular strength and vibration thresholds during two years after acute poisoning with organophosphate insecticide. . *Journal of Ocupational & Environmental Medicine.,* , 61:1-6.

MoA. (2013). *Ministry of Agriculture and rural development annual report.* Addis Ababa: Ethiopia.

Monaco, J., Weller, S., & Ashton, F. (2002). Herbicide registration and environmental impact. In Weed Science: Principles and Practices, 4th ed.; Monaco, T.J., Weller, S.C., Ashton, F.M., Eds.; John Wiley & Sons: New York, NY, USA, .

Mugachia, J. C. (1992). Organochlorine pesticides in Estuarine fish from the Athi River, Kenya. *Bulletin of Environmental Contamination and* , 49(2): 199-206.

Muli et al. (2014). Evaluation of the distribution and impacts of parasites, pathogens, and pesticides on honey bee (Apis mellifera) populations in East Africa. *PLoS One.* , 9(4):e94459.

Namies´nik:, M. W. (2012). Application of dispersive liquid–liquid microextraction for the determination of selected organochlorine pesticides in honey by gas chromatography–mass spectrometry: Eur Food Res Technol 234:.

New Zealand Food SafetyAuthority. ((2010).). *Background on toxic honey [online].http://www.nzfsa.govt.nz/animalproducts/publications/info-pamphlet/bee-products/toxic-honey.htm.[Accessed 2016-11-12].*

Pereira, J., Antunes, S., Castro, B., Marques, C., Gonçalves, A., & Gonçalves, F. (2009). Toxicity evaluation of three pesticides on non-target aquatic and soil organisms: Commercial formulation versus active ingredient. *Ecotoxicology , 18,* , 455-463.

Picó, Y. F. (2007). Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment. J. Biochem. Biophys. Methods 70, . 117–131.

Reus et al, .. (2002). Comparison and evaluation of eight pesticide environmental risk indicators developed in Europe and recommendations for future use. *Agr.Ecosyst. Environ. , 90*, 177-187.

Rissato S.R., G. M. (2007). Multiresidue determination of pesticides in honey samples by gas chromatography-mass spectrometry and application in environmental contamination. Food Chem., 101, 1719.

SEBLEWORK, e. a. (2014). 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,* , Vol. 33, No. 6, pp. 1294–1302, .

Shenkute et al. (2012). Honey production systems (Apis mellifera L.) in Kaffa, Sheka and Bench-Maji zones of Ethiopia. *ournal of Agricultural Extension and Rural Development , Vol. 4(19)*, pp. 528-541.

TADESSE, & ASFERACHEW. (2008). AN ASSESSMENT OF THE PESTICIDE USE, PRACTICE AND HAZARDS IN THE ETHIOPIAN RIFT VALLEY. *PESTICIDE ACTION NETWORK(UK)* , 25-30.

United States Environmental Protection Agency. ((2010).). *About pesticides [online]. http://www.epa.gov/pesticides/about/.* [Accessed 2016-11-04]: EPA.

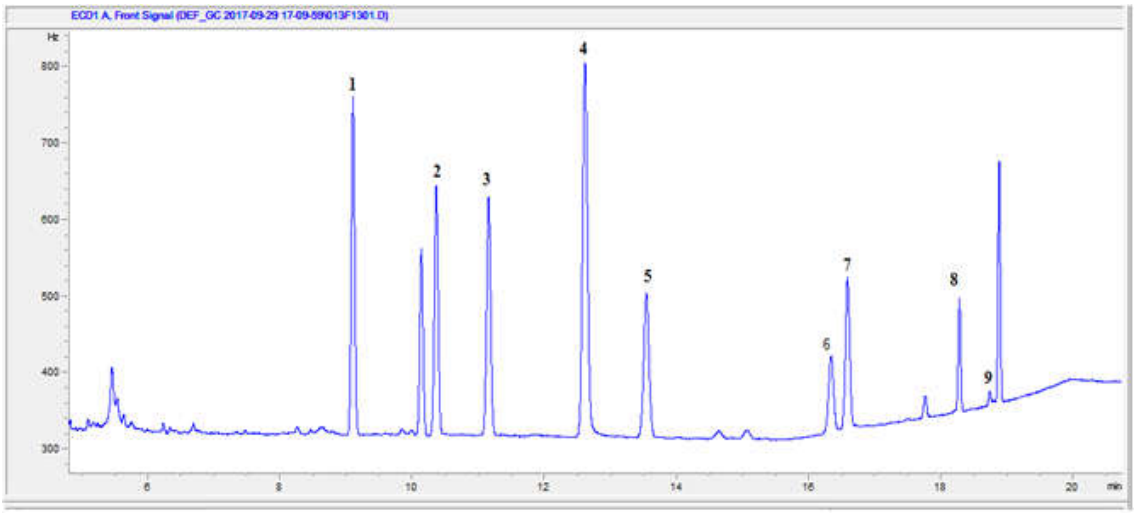
Wang J., K. M. (2010). Residues of organochlorine pesticides in honeys from different geographic regions, Food Research International, 43 (9): 2329–2334.

Wiest L., B. A. (2011). Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography ccoupled with mass spectrometric detection. J. Chromatogr. A, 1218,5743.

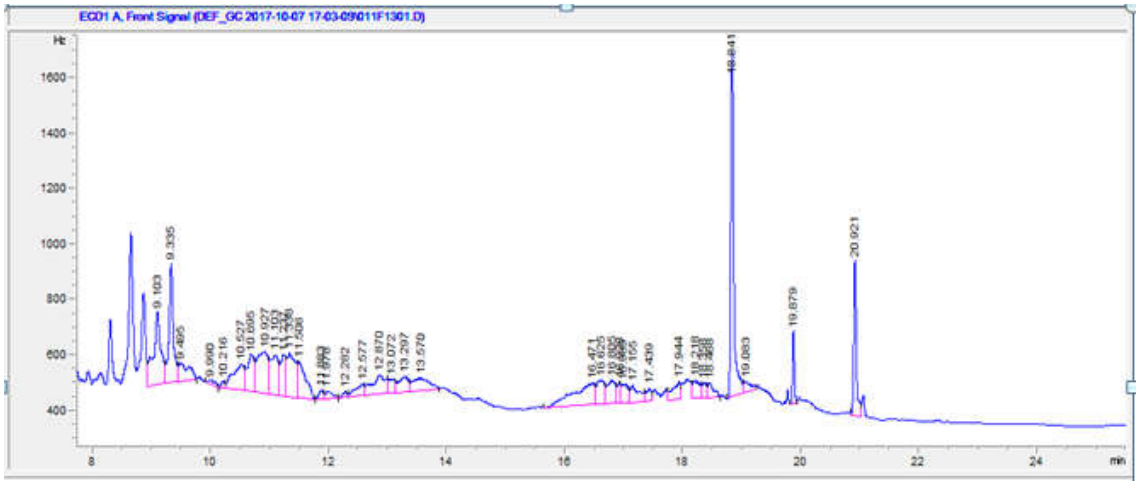
ZZhang, W. J. (2011). . Global pesticide consumption and pollution . *China as a focus* , 125–144.

Annexes

Annex I: Standard chromatographic picture



Annex II: Honey sample chromatographic picture



Annex 3: Photographic images of honey sample collection