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THESIS ON

DETERMINATION OF ORGANOCHLORINE PESTICIDE RESIDUE LEVELS IN CHEWABLE AND LEFTOVER PARTS OF THE KHAT PLANT (*Catha edulis*) FROM JIMMA ZONE, SOUTHWEST ETHIOPIA

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DETERMINATION OF ORGANOCHLORINE PESTICIDE RESIDUE LEVELS IN CHEWABLE AND LEFTOVER PARTS OF THE KHAT (*Catha edulis*) PLANT FROM JIMMA ZONE, SOUTHWEST ETHIOPIA

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MSc THESIS APPROVAL SHEET

We, the undersigned, member of the Board of Examiners of the final open defense by **FITSUM TAMIRAT** have read and evaluated his/her thesis entitled "**DETERMINATION OF ORGANOCHLORINE PESTICIDE RESIDUE LEVELS IN CHEWABLE AND LEFTOVER PARTS OF THE KHAT (*Catha edulis*) PLANT FROM JIMMA ZONE, SOUTHWEST ETHIOPIA**" and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree Master of Science in **Applied Chemistry (Environmental Chemistry)**.

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List of abbreviations

BQL	Below quantification limit
CNS	Central nervous system
d- SPE	Dispersive Solid-Phase Extraction
DDD	Dichlorodiphenyldichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic Acid
DSM-IV	Diagnostic and Statistical Manual
EC	European Commission
EPA	Environmental Protection Agency
EU-MRL	European Union Maximum residue limit
FAO	World Food Organization
FDA	Food and Drug Administration
GC	Gas chromatography
GCB	Graphitized carbon black
GC-ECD	Gas Chromatography Electron Capture Detector
GC–MS	Gas Chromatography–Mass Spectrometry
GHS	Globally harmonized System
HCH	Hexachlorocyclohexane
HPLC	High Performance Liquid Chromatography
IVOMD	In *vitro organic matter digestibility*
JMPR	Joint Meeting on Pesticide Residue
LC	Liquid chromatography
LCC	Lower Calibration Curve
LC–MS	Liquid chromatography coupled mass spectrometry
LD	Lethal Dose
LD_{50}	Lethal Dose
LLD	Liquid–Solid Extraction
LLE	Liquid–Liquid Extraction

LOD	Limit of detection
LOQ	Limit of quantification
MoFED	Ministry of Finance and Economic Development
MRL	Maximum Residue Level
MRLs	Maximum Residue Levels
MSD	Mass Selective Detector
MSPD	Matrix Solid-Phase Dispersion
OCP	Organochlorine pesticide
POP	Persistent Organic Pollutants
PSA	Primary secondary amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, Safe
RSD	Relative standard deviation
SIM	Selected Ion Monitoring
SNNP	South Nationality of People
SRM	Selective response monitoring
UK	United Kingdom
US	United States
USA	United States of America
WHO	World Health Organization

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Abstract

Khat is one of the most consumed plants in the horn of African countries. However, it is a stimulant plant that has several side effects on the health of consumers. In addition, the fresh Khat (tender leaves) used for human consumption and the leftover part used as feed for goats and sheep often contain contaminants such as pesticide residues. The main objective of this study was to determine the concentrations of selected organochlorine pesticides in chewable and leftover parts of Khat from the Jimma zone, southwest Ethiopia. A total of 120 Khat leaves were sampled, 60 of which were chewable and purchased from local markets in Jimma city. The other 60 mature leaves were leftover Khat samples collected from three predominantly Khat-producing districts in the Jimma zone. A newly modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) method was used for the extraction of the pesticides from both the chewable and leftover Khat samples. The method was validated before it was applied. Accordingly, the limit of detection (LOD), limit of quantification (LOQ), repeatability and reproducibility, and percentage recovery were determined. Target analytes were determined using GC–MS. The spiked recovery results for eight analytes were within the acceptable range of the European Union SANCO. The study revealed that both the chewable and leftover Khat samples were contaminated by two types of OCPs out of eight target analytes. Accordingly, p,p'-DDE and p,p'-DDT were detected in 88.3% and 76.6%, respectively, of the chewable samples and in 86% and 69%, respectively, of the leftover Khat samples. The concentrations of p,p'-DDE and p,p'-DDT in chewable Khat were in the range of 34.07–45.39 and 11.17–34.15 µg/kg, respectively, and the concentrations of p,p'-DDE and p,p'-DDT in leftover Khat were in the range of 30.12–45.04 and 22.21–32.16 µg/kg, respectively. A high concentration of the metabolite (p,p'-DDE) compared to the precursor compound (p,p'-DDT) revealed the historical use of DDT in the study area. The concentrations of pesticides did not vary significantly between the sample sources (P = 0.823). Among the detected analytes, none were above the MRL values set by the FAO/WHO or the European Commission (EC). However, the chewable and leftover Khat available for consumption were contaminated with p,p-DDT and its principal metabolite p,p-DDE. Thus, the cumulative effect and synergistic toxic effect of these pesticides on Khat Consumers should be considered. Therefore, all the bodies of concern in the country need to play the crucial role of ensuring that the Khat consumed by humans and animals is not a health concern.

Keywords: *QuEChERS extraction method; OCPs; DDT; p,p'-DDE Catha edulis; GC–MS*

1. INTRODUCTION

Pesticides are frequently used in agriculture to manage harmful pests, including insects, plant diseases, weeds, and other living organisms, that risk our food supply and health during food production, processing, storage, transport, and marketing.[1,2] Despite their uses, pesticides may have an impact on human health and the environment due to residues that persist in food and environmental samples.[3] As a result, contamination of food products with such toxic compounds is a global public health concern.[4] There are numerous agricultural pesticides on the market today, most of which are phenoxy compounds, carbamates, or organochlorines. In addition to being poisonous, organochlorine pesticides also have a lengthy half-life and do not degrade easily.[5]

Among others, the pesticides dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) are examples of organochlorine pesticides (OCPs) that offer competitive advantages for agricultural productivity. However, due to their toxicity, persistent nature, high biomagnification potential, and harmful effects on human health, DDT and HCH have been banned from use in agriculture since the 1980s, and they are classified as persistent organic pollutants (POPs) by the Stockholm Convention.[6] Ethiopia joined the list of signatories to this agreement.[7] Generally, oxidative stress is caused by organochlorine pesticides because they increase the body's production of free radicals while lowering the action of antioxidant enzymes. DDT was one of twelve "persistent organic pollutants" (POPs) that were prohibited by the agreement.[8] Organochlorine compounds have been found to be present in water, soil, sediment, fish, meat, honey, vegetables, and fruits according to various studies.[9–17] However, there are very few studies on OCP residues in Khat plants, which are widely consumed in Ethiopia. Khat is an evergreen, blooming tree with mild narcotic properties that is most frequently observed in East Africa. It was first raised in Ethiopia and Yemen. Khat leaves are chewed daily as a stimulant by five to ten million individuals, mostly in the Middle East and Eastern Africa.[18,19]

The plant is forbidden in several western countries, but it is legal in Djibouti, Kenya, Ethiopia, Somalia, and Yemen. Khat is a highly valued export item from Ethiopia.[20] The overall pooled incidence of Khat use was 16.7% (13.7–19.7%). The pooled prevalence of Khat use was different across regions; it was highest in the Oromia region (21.10%; 15.52, 26.68), and we found similar prevalence rates in the Amhara and SNNP regions (14.78 [10.6, 18.9] and 14.3 [10.3, 18.3], respectively).[21] In addition to human consumption, many studies have shown the importance of Khat as a source of animal feed in Ethiopia.[22–24]

According to S El-Zaemey et al.[25] and his colleagues, Ethiopian Khat farmers, used DDT and other pesticides as chemicals for Khat growth. Moreover, only a small number of studies that found exceptionally high levels of p,p'-DDT in Khat samples collected in the eastern part of Ethiopia, ranging from 141.2 to 999.0 µg/kg, have been reported.[26] Another study performed in the southern region of Ethiopia revealed that residual p,p'-DDT levels in Khat samples might reach 44.8 µg/kg.[27] In the chewable parts of Khat, where the current study was being undertaken, substantial amounts of total DDT were also discovered in one prior examination in the Jimma Zone, Southwest Ethiopia, ranging from 41.24 to 148.87 g/kg.[28]

Jimma zone is one of Ethiopia's Khat-growing regions and is located in the country's southwest, where the majority of people have a long-standing tradition of chewing Khat.[29] Fojo, G. A. and Tesfa, A.[30] reported that Khat is chewed among different religious followers; however, it is chewed more among Islamic religious followers than among others. This is because the majority of the inhabitants in the Jimma districts where this study was undertaken are Muslim religion followers, and some Muslims believe that Khat use has religious significance.

Farmers in southwest Ethiopia also use DDT on the leaves of Khat plants to keep them shiny and attractive to buyers. Organochlorine pesticides such as DDT have been linked to various health problems, including cancer, reproductive and developmental disorders, and neurological effects. Knowing the concentration of DDT and its metabolites in the fresh tender section of the plant consumed by humans and in the leftover section of the plant consumed by animals, especially sheep and goats, is essential because it can help to identify the potential risks associated with consuming Khat contaminated with DDT and its metabolites. Humans commonly consume the fresh tender section of the plant without any processing, while the leftover section is often fed to animals such as sheep and goats. If the levels of DDT and DDT metabolites in these parts of the plant exceed the maximum residue limits of the European Union Regulation Guidelines in Animal Feed (CE396/2005), they could pose a risk to human health through direct consumption or through the consumption of meat from animals that have been fed contaminated Khat. Therefore, determining the concentration of these pesticides in both parts of the plant is crucial for assessing the potential risks to human and animal health and for developing appropriate measures to ensure food safety.

Consequently, the purpose of this study was to determine the levels of organochlorine pesticides in chewable Khat samples collected from local markets in Jimma city. However, four districts (Kersa, Seka, Dedo, and Mana) and one kebele (Jiren) of Jimma Zone, which are considered as a source of chewable Khat for the local market. Additionally, leftover Khat samples obtained from three predominant Khat producing districts in the Jimma zone, namely, Seka, Mana and Kersa, by using a newly modified QuEChERS sample extraction procedure followed by GC–MS detection.

1.2 Statement of the problem

Approximately 1.8 billion people are estimated to be employed in agriculture globally today, and the majority of them use pesticides to control pests in food and other commercial products.[31] Ethiopia's current development initiatives involve intensifying agricultural production on both a small scale and commercial scale. It is thought that Ethiopia is using alarmingly more pesticides, both in terms of type and quantity.[32] The misuse of pesticides was a common problem mainly because farmers lacked the necessary understanding of pesticides and because ineffective administrative controls limited their usage.

Several studies have been performed by different scholars on the determination of pesticide residue in Khat grown in Ethiopia.[33–37] Although, different studies have determined the level of organochlorine pesticides in Khat, some gaps have not yet been widely assessed. This study identified the following gaps in recent studies that were carried out to determine the level of OCPs using only the Khat part, which is consumed by humans; the authors did not pay attention to the OCPs in the Khat part, which are eaten by animals that are used as food for humans, such as goats and sheep. Furthermore, when organic matter is dumped as waste and enters the soil in the form of compost, the amount of soil OCPs increases. Literature gaps for the current status of OCP residue levels were considered. However, before seven years, the findings of Mekonen et al.[28] showed the presence of very high OCP residues compared with those of MRLs set by FAO/WHO and EC. However, the current status of the OCP residue level in Khat has not yet been studied at the current study site in the Jimma zone, southwestern Ethiopia. Therefore, it is imperative to determine the level of OP residues in the chewable and leftover parts of Khat using a newly modified QuEChERS method followed by GC–MS analysis.

1.3 Objective of the study

1.3.1 General objective

- The main objective of this study was to determine the concentrations of selected organochlorine pesticides in chewable and leftover parts of Khat collected from the Jimma Zone, Southwest Ethiopia.

1.3.2 Specific objective

The specific objectives of this research are as follows:

- To determine the levels of eight organochlorine pesticide residues in chewable and leftover Khat samples
- To compare the residue levels in chewable and leftover Khat samples with the International Maximum Residue Level (MRL)

1.4 Significance of the study

This study focused on the presence of organochlorine residues in the chewable and leftover parts of Khat collected from selected districts and local markets in Jimma city, southwest Ethiopia. The results of the study will have the following significance:

- The consumers will have awareness about the level of the selected organochlorine pesticide residue in the Khat samples at the selected sites.
- The "determination of the level of Organochlorine pesticide in leftover parts of Khat that is used as feed for goats and sheep" could ensure the safety and health of the animals consuming it.
- The findings of the study will help regulatory bodies and other stakeholders establish MRLs and implement monitoring programs.
- This study provides additional information on the impact of the selected organochlorine pesticides on the study sites.
- This study provides into insight how organochlorine pesticides can be managed and used safely.
- The findings of the study will serve as baseline reference material for further study.

1.5 Research questions

1. What is the level of selected organochlorine pesticide residues in chewable Khat samples?
2. What is the level of selected organochlorine pesticide residues in leftover Khat samples?
3. Do the residue levels in chewable and leftover Khat samples exceed the international maximum residue level (MRL)?

2. REVIEW OF THE LITERATURE

2.1 Khat (*Catha edulis*)

Khat is an evergreen perennial shrub plant that belongs to the Celastraceous family. There are several names for the plant, depending on its origin: chat-Ethiopia, qat-Yemen[38,39], qaad/jaad-Somalia[40], qu`t, Catha, gat, tohat, and muraa.[41] The dried leaves of Khat are known as Abyssinian tea or Arabian tea.[42]

Khat (Catha edulis Fork), a mild stimulant consumed by chewing, is a psychoactive shrub or plant chewed for its stimulating effects. It is a species belonging to the kingdom Plantae family Celastraceous. Although the birth of the Khat tree is contested, many believe that it originated in Ethiopia.[43] People in East Africa and the Arabian Peninsula chewed the leaves of Catha edulis for their stimulant effects. Reports from experts on Khat use in the hinterlands of the Horn of Africa argue that the consumption of Khat goes back at least eight centuries. For instance, the leaves were chewed by people who lived in the medieval Islamic sultanates of the southern region in what is currently known as Ethiopia as early as the 14th century. [44,45]

2.1.1 Soils and topography for Khat

Khat performs best on soils with a pH ranging from 6.0-8.2. Nevertheless, once established, Khat grows well under a wide range of soil types and climatic conditions. The optimal altitude for growing Khat ranges from 4 to 1500-2100 m. In Ethiopia, Khat is extensively grown and thrives best in the mid-land (1500-2500 m), but it can also be cultivated with irrigation down to 1000 m if the area is free of frost. At the early development stage, the water supply is more critical than the soil type. The field should be well managed and drained for good crop performance.[39]

2.1.2 Production and consumption of Khat in Ethiopia

In contrast to the rainy season, from April to September, when demand is high, Khat is more expensive when it is dry. The Ethiopian government does not support the production of Khat in any way and does not take any action to prevent its growth, trade, or use. Khat currently occupies 94,330 hectares of land in the country or roughly one-third of the area covered by coffee.[46] The majority of the Khat produced for both the domestic market and exports is made in Hararghie.

According to research performed in Hararghie by Getachew, M[47] from the end of the 19th century until the 1940s, the primary goal of Khat production was to meet local demand. Historically, Muslims have used Khat for religious activities, such as reading the Koran and praying.[47] The oldest Khat plantation among the houses studied, according to the farmers, was planted in the 1920s, revealing that Hararghie has a long history of using this drug. However, due to easier access to transportation and greater market opportunities, Khat, a commercial crop, started farming earlier in eastern Hararghie than in western Hararghie. According to the farmers, cultivation as a cash crop started in western Hararghie (specifically in Habro) in the 1970s. Khat production has increased since 1970, as indicated by assessments in the area. Before 1970, the farmers studied had just approximately 5 hectares of land under cultivation; today, 34 hectares of this area are used for Khat cultivation. Approximately 70% of the farmers were growing Khat between 1980 and 1990, but currently, all of the farmers polled are doing so. There are currently very few non-Khat growers among the farmers in the research region. Khat cuttings are typically planted initially in the vicinity of the homestead, after which they spread outward to cover the remaining field. In the 2 examined locations, Khat accounts for 55% of the cultivated land. [47]

2.1.3 Use of Khat

Economic use: In Ethiopia, Khat is an important and potentially profitable cash crop. The employment opportunity created through the cultivation of Khat is very high because a large number of people are involved in growing, harvesting, sorting, packing, transporting, loading and unloading the commodity. The wood of the plant is commonly used for fuel, and due to its termite resistance, it is used in the construction of houses and fencing. It is also used for making rafters; handling farm tools (hammers and chisels); handling household articles such as pots and pans; rolling pins; and making forks, combs, spoons and rulers.[48]

Medicinal use: Processed leaves and roots are used to treat influenza, cough, gonorrhea, asthma and other chest problems. The root is also used for stomach ache, and an infusion is taken orally to treat boils.[49] Khat has considerable social value. It is used to welcome and entertain guests in mourning, weddings and circumcision ceremonies and collective labor works. Khat chewing is associated with ceremonies such as smoking incense, smoking cigarettes and drinking drinks (soft drinks, tea and milk).[50]

Environmental value: In Ethiopia, Khat is grown in an intensive production system. It is planted in rows on hillsides along terraces in association with different food crops, mainly annuals, and is oriented against slopes. As such, Khat cultivation plays a key role in controlling soil erosion, which is a major threat in most areas of Ethiopia due to the undulating topography and intensive deforestation for farmland expansion; hence, Khat culture is considered to be the best agroforestry system practiced by farmers. Had this not been the case for the cultivation of Khat, the erosion of topsoil would have been severe and possibly disastrous in a few areas of Ethiopia.[39]

2.1.4 Export capacity of Khat in Ethiopia

Ethiopia is the world's largest producer of Khat, which is also the fastest-growing export country. The majority of the crop is sold and consumed domestically, primarily in the Somali administrative zone, with approximately one-third of it being shipped to Djibouti and Somalia.[51] Khat was Ethiopia's second-largest export in 1998–1999, making up 13.4% of the country's total export revenue in that year.[52] As Saha, S. and Dollery, C[53] reported, Ethiopia exported only 16% (22,400 tons) of the total production (136,802.73 tons). The remaining 31 and 53 percent are consumed by producers and nonproducers, respectively, of Khat domestically. This shows that 84% of the total production is consumed in the domestic market, and if this is exported, the country would generate approximately 568 million US dollars.

Despite the scarcity of available national data, the Ethiopian government reaps substantial profits from the Khat export tax. The west Hararghie finance office states that Khat export is taxed at 6 birr/kg, while domestic Khat is taxed at 3 birr/kg. A study conducted by the US Embassy also validated this level of export tax.[52] Khat's price varies seasonally based on the quality and quantity of the supply. According to Khat exporters, Jigjiga purchases of export quality Khat cost 3-7 birrs (US$0.36-0.85) per kg, whereas Djibouti purchases cost 8-30 birrs (US$0.97-3.62) per kg.

2.1.5 Khat chewing

Fresh leaves from Khat trees are chewed daily by more than 20 million people in the Arabian Peninsula and East Africa.[50,54] Recent reports suggest that 80–90% of male adults and 10–60% of female adults in East Africa consume Khat daily.[55] New patterns of Khat consumption, including morning chewing sessions and Khat parties, have emerged in these East African countries.[55] Traditionally, Khat has been chewed by Muslims during religious ceremonies and during prayer to facilitate contact with Allah.[56] Today, chewing is common among people of other religions and in most parts of the country.[48] A recent survey of one region in rural Ethiopia placed the prevalence of current use at approximately 50% of the total population.[57] Some of the effects of Khat chewing include enhanced concentration, feelings of euphoria and suppression of hunger and sleep. Khat is also chewed to increase work efficiency and facilitate social interactions.[49] The effect of Khat varies from person to person. Some people mention that the main reason for chewing is an enhancement of socialization and that ceremonies are a nice way of spending time with friends, not as much as the physical effect of Khat, such as staying awake or getting "high". Different production areas and varieties of Khat have been reported to have dissimilar effects on the same chewer.[48] The active ingredient in Khat is the alkaloid cathinone, sometimes called "natural amphetamine".[58] Khat is preferably chewed within two days after harvesting, after which the leaves lose much of the desired material.

Khat has a long history of medical use, and in the Harare region, people consider Khat to treat more than 500 ailments. Historically, Khat has also been used as medicine to treat symptoms of depression and melancholia, and sources tell about Alexander the Great using Khat to cure ill soldiers.[59] In the eastern parts of Ethiopia, Khat is known to treat influenza as well as coughs and asthma.[49] The effect of Khat chewing varies according to the type of Khat and the person. For example, the euphoric, cheerful sensation and excitement stage lasts approximately 1–2 hours.[60,61]

2.1.6 Dependence of Khat

Khat often starts to be used at a young age, can develop into a compulsive daily habit that lasts a lifetime and is practiced by both men and women.[62,63] Khat-taking behavior depends not only on the reinforcing psychostimulant action of Khat but also on deeply rooted cultural factors.[63] Khat is commonly consumed, as indicated by the tendency of Khat chewers to secure their daily supply at the expense of vital needs and their behavior at markets.[63,64] This is described as a psychological dependence by many authors.[65]

In eastern African countries, the prevalence of chat dependence is estimated to be 5–15% of the population.[63] Giannini reported two cases of Khat addiction that were effectively treated with bromocriptine using a protocol developed for cocaine addiction.[66] From their description, it could be inferred that both of these patients satisfied the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV).[67] criteria for substance abuse. Khat is postulated to have greater dependence potential than amphetamine[65] because of its less aversive nature[68], higher rate of self-administration, and rapid onset of action in discrimination experiments[69] compared to amphetamine in various operant experiments.

Although cathinone is known to cause sensitization upon repeated administration, similar to amphetamines, there are reports of tolerance to the CNS-stimulating effects of chat chewing. This is alleged to be due to the physical limits on the amount that can be chewed rather than the inherent property of Khat leaves.[70] There are conflicting opinions regarding the existence of a withdrawal syndrome. Generally, it is believed that there is no physical withdrawal syndrome as experienced with alcohol, morphine or barbiturates.[71,72] Abandoning the Khat chewing habit, however, is followed by symptoms such as lassitude, anergia, nightmares, slight trembling, and depression.[40,57] However, there are reports of social withdrawal symptoms after cessation of the habit, described as "experiences of deprivation of joys and camaraderie which Khat session almost unfailingly provides". As in the case of drug abuse in industrialized societies, Khat use is associated with the simultaneous use of other drugs, especially cigarettes and alcohol.[73]

2.1.7 Legal considerations

Khat circulates freely in Yemen, Ethiopia (despite the Orthodox Tewahdo Church prohibiting its use), Somalia (although briefly banned during the six-month rule of the United Islamic Courts in Mogadishu in 2006) and some other East African countries.[57] Almost every small kiosk in Addis Ababa, the Ethiopian capital, openly sells Khat, and in small cities and towns all over the country, it is brought to market as produce, where people publicly chew it and offer it to visitors as a mark of hospitality.[74] In Yemen and Ethiopia, there have been attempts to curtail these habits for social and economic reasons, but these attempts have met with little success.[40,75] One reason for this is that in Yemen, and in some parts of Ethiopia, it is consumed by government officials, making its regulation very difficult.[50] Although the active alkaloids of Khat, namely cathinone and cathine, have been labeled Schedule I and Schedule III, respectively, by the WHO since 1971, their status in European countries has not been uniform.[65] For example, Khat is prohibited in Ireland, France, Switzerland, Sweden and Norway[54,61], while it is legal in the U.K. and the Netherlands[76,77] Outside of Europe, it is illegal in the U.S.A. and Canada but permissible in Australia.[54] Recently, the WHO Committee reviewed the data on Khat and determined that the potential for abuse and dependence is low and that the threat to public health is not significant enough to warrant international control; additionally, the WHO Committee did not recommend the scheduling of Khat.[78]

2.1.8 The toxicological potential of Khat

Chewing fresh leaves is a widespread habit in local populations, with several million people consuming Khat regularly in social sessions that often last for hours.[50] Khat users reported increased levels of energy, alertness and self-esteem; a sensation of elation; enhanced imaginative ability; and a greater capacity to associate ideas. These effects have been attributed to Khat's content in cathinone, a sympathomimetic amine with properties similar to those of amphetamine.[79] Other less potent stimulant substances may also be present, namely, nor-pseudoephedrine (cathine) and nor-ephedrine.[50] Like that of amphetamine, the pharmacological profile of cathinone, has been shown to result from the increased release of monoamine neurotransmitters from nerve terminals.[64] Considering the structural and pharmacological similarities between cathinone and amphetamine, it might be expected that the toxic effects could also occur.

In humans, adverse effects common to those of amphetamine have also been observed in Khat users, namely, a depressive stage, which usually occurs at the end of the Khat session (which depends on the potency of the Khat), mydriasis, irritability, anorexia, hyperthermia, insomnia and endocrine disturbances. The "next morning" set of symptoms, which include lethargy, a sleepy state and a crave for Khat leaves, are also characteristic.[50] The oral consumption of Khat by people on the Arab Peninsula has been linked to esophageal carcinoma.[80] Accordingly, human genotoxicity caused by Khat chewing was ascertained by the occurrence of micronuclei in exfoliated buccal and bladder cells of Khat consumers.[81] Chronic use of Khat has also been associated with the development of hypertension, psychosis, cognitive impairment and disabling neurological illness[82], as well as an increased incidence of acute coronary vasospasm and myocardial infarction.[83] The possible effects of Khat consumption-induced sustained oxidative stress may lead to oxidative damage to cellular macromolecules such as DNA, lipids and proteins, contributing to the development of several pathologies, notably cancer, hepatotoxicity, nephrotoxicity, cardiovascular toxicity and neurodegenerative diseases.

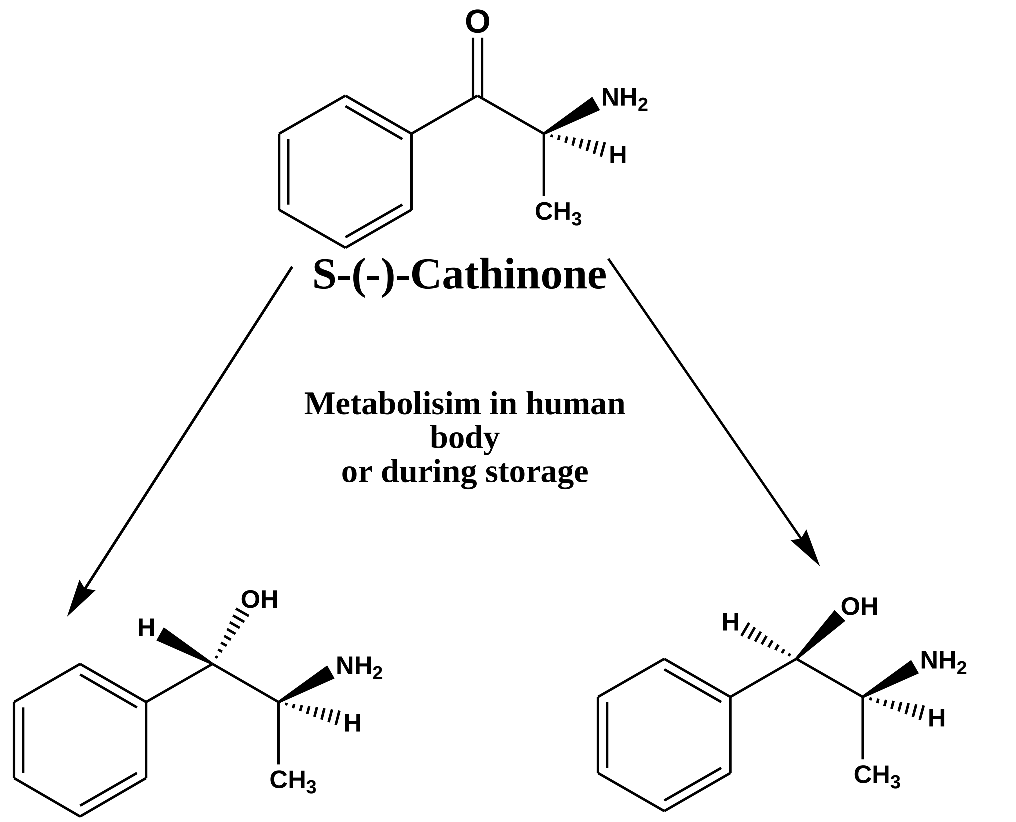
The most obvious effect of Khat-use was observed on the digestive system, where gastritis is fairly common among regular chewers due to the effects of the acid tannins in Khat. Daily intake can also cause chronic constipation. Khat fever, insomnia and hypertension are other side effects of Khat, as are dental and oral problems.[40] Khat is said to have reproductive toxicity in humans, and several studies have shown that Khat use can lead to a decrease in sperm quality, low birth weight and lactation inhibition in mothers.[45]

2.1.9 Chemical aspects of Khat (*Catha edulis)*

Early interesting work was performed on the Khat plant, during which the main psychoactive compound, cathinone, was discovered. The leaves and roots of this plant consist of many compounds that belong to different compound classes, such as the alkaloids of phenylalkylamines (phenylpropylamines and phenylpentenylamines), cathedulins, flavonoids, sterol and triterpenes, monoterpenes and volatile aromatic compounds, and other miscellaneous compounds, such as amino acids and vitamins.

Phenylalkylamines and cathedulins are the major alkaloids. At the UN Narcotics Laboratory, together with Schorno and Steinegger at the University of Berne, Switzerland, the authors isolated and identified the phenylalkylamine, (−)-α-aminopropiophenene, which was later named S-(-)-cathinone, as a major active constituent in fresh Khat.[84] The plant contains the (–)-enantiomer of cathinone only[78,85], which has the same absolute configuration as S-(+)-amphetamine.[65,86] As shown in Figure 1 during maturation, cathinone is enzymatically converted to cathine [(+) or pseudoephedrine] or (−)-norephedrine.[87] Sunlight-induced or heat-induced degradation of cathine and norephedrine also occurs during the extraction of cathinone in the laboratory.[88] Indeed, to slow the degradation process, Khat leaves are usually wrapped in banana leaves immediately after picking to retain their moisture.

Other alkaloids of phenylalkylamines in Khat leaves include the phenylproanolamine diastereoisomers of cathine [1S, 2S-(+) norpseudoephedrine or (+) norpseudoephedrine] and norephedrine [1R, 2S-(−) norephedrine]. Cathine and norephedrine occur mainly in older plants and are also formed by the reduction of cathinone during drying and storage. Cathinone is transformed mainly to cathine in Khat leaves and mainly to norephedrine by human metabolism (see Figure 1).[89] The environment, climate conditions, and local traditions associated with cultivation and harvesting determine the chemical profile and general appearance of Khat leaves.[90] The phenylalanine content of Khat leaves varies widely. In certain Khat samples, the phenyalkylamine fraction consisted of up to 70% of the (−) cathinone, and the (−) cathinone content was correlated with the market price of Khat.[91]



S, S-(+)-Norpseudoephedrine (Cathine) **R, S- (-)-Norephedrine.**

Figure 1: Chemical structures of S-(−)-cathinone, S, S-(+)-norpseudoephedrine (cathine) and R, S- (−)-norephedrine.

2.1.10 Harvesting

From planting a stand of Khat to regular harvesting normally takes 2 to 3 years though state five to eight years, although it takes five to eight years for the tree to reach maturity.[92] A healthy tree will continue to give good harvests for another 50 years. Khat is not damaged to any extent by any known pathogens.[93] Farmers of this crop consider the presence of a tiny green leaf hopper (Empoasca species) to be beneficial because it causes older tips to wilt and die off for the eventual emergence of new shoots, which can be harvested. However, before harvesting, the hopper is removed by dusting the plants with very fine dust particles. This approach appears to be effective at preventing the hopper from attaching itself to the tree. Recently, some farmers have used an insecticide to eliminate hoppers. The compound is mixed with plant juice, which is consumed by the hopper. Although the use of insecticides leads to improved branching and leaf growth, there are concerns related to their safety. Indeed, many consumers look for Khat bundles that have not been treated with insecticides. As a result, many farmers are returning to fine dust treatment to achieve customer satisfaction. Khat is harvested by breaking off the young branches from the main branches and trimming them to approximately 40 cm. Depending on the growth stage of the harvestable products, there are different types of Khat products. Young and soft shoots are detached with the bare hands, while hard shoots are cut off by hand tools. Each harvestable product is locally given name (s). Khat has a short shelf life and cannot be kept for longer than 2-3 days. Mature Khat plants should be harvested and marketed without delay; otherwise, their quality deteriorates, and they lose market value.[50]

2.1.11 Processing methods and marketing

Several procedures and processes are employed to ensure the marketable value of the harvested material from Khat. The consumable part is harvested and put in shawls or plastic sacks at the farm level and taken home for sorting and grading by removing the leathery leaves and trimming the long stems. The selected material and the unfit/unmarketable portion, locally called garaaba, are separated. The unfit part is set aside for animal feed and as compost material for later use as manure. The selected and marketable part was tied into a haqara/bundle (40-60 selected slender twigs) and splashed with water to keep the product wet and fresh. The plants were subsequently wrapped with fresh leaves and twigs from different plants and grasses.[39] At best, the leaves can remain in an acceptable condition for up to 5 days.

Figure 2: Photo of Khat wrapped with fresh false banana leaves

However, it is an important commercial fact that the value of the leaves drops dramatically after the first day of harvesting. This reflects the instability of the main active constituents in the isolated leaf, which was appreciated by the consumer a long time before the scientist.[94] (-)-S-Cathinone, the main effective constituent of Khat leaves, is relatively unstable and decomposes into (-)-norpseudoephedrine and norephedrine within a few days of picking or if the leaf is dry. Thus, the treatment of only freshly picked leaves has full efficacy. Norpseudoephedrine and norephedrine are slowly absorbed and then excreted mainly unchanged within approximately 24 h.[95] The major metabolites of (-)-S-cathinone after ingestion are (-)-R, S-norephedrine and (-)-R, Rnorpseudoephedrine.[95]

2.2 Pesticides

2.2.1 Definition of pesticides

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

2.2.2 Classification of pesticides

Pesticides are classified based on various criteria, such as toxicity (hazardous effects), pest organism they kill, pesticide function, chemical composition, mode of entry, mode of action, how or when they work, formulations and sources of origin.

I. Classification of pesticides based on toxicity

The toxicity of pesticides mainly depends on two factors, namely, dose and time. Hence, how much of the substance is involved (dose) and how often the substance is exposed (time) give rise to two different types of toxicity—acute and chronic toxicity.[96,97]

Acute toxicity- Acute toxicity refers to how poisonous a pesticide is to a human, animal, or plant after a single short-term exposure. A pesticide with high acute toxicity can be deadly even when a very small amount is absorbed. Acute toxicity may be measured as acute oral toxicity, acute dermal toxicity or acute inhalation toxicity.

Chronic toxicity- Chronic toxicity refers to the delay of poisonous effects from exposure to a pesticide. The chronic toxicity of pesticides concerns the general public as well as those working directly with pesticides because of potential exposure to pesticides on/in food products, water and the air.

The World Health Organization (WHO) has highlighted only acute toxicity for the classification of pesticides. According to the WHO, pesticides are classified by acute oral and acute dermal toxicity using the estimated lethal dose (LD50) (the pesticide dose that is required to kill half of the tested animals when entering the body by the oral or dermal route). At present, the widely used 'WHO recommended classification of pesticides by hazard' suggests allocating pesticides to 'the specific WHO hazard classes'. After revision in 2009, these classes were harmonized with the 'Globally Harmonized System (GHS) Acute Toxicity Hazard Categories.[98]

II. Classification of pesticides based on chemical composition

This approach is the most common and useful method for classifying pesticides based on their chemical composition. Pesticides such as insecticides, fungicides, herbicides and rodenticides are also classified based on their chemical composition as follows:

Insecticides: Based on their chemical composition, insecticides are classified as carbamates (carbaryl), organochlorine (Endosulfan), organophosphorus (monocrotophos), and pyrethroids (permethrin); neonicotinoids (imidacloprid); or miscellaneous pesticides such as spinosyns (spinosad), benzoureas (diflubenzuron), and antibiotics (abamectin). Insecticides are important pesticides that can be further classified into several subclasses (Figure 3).[99]

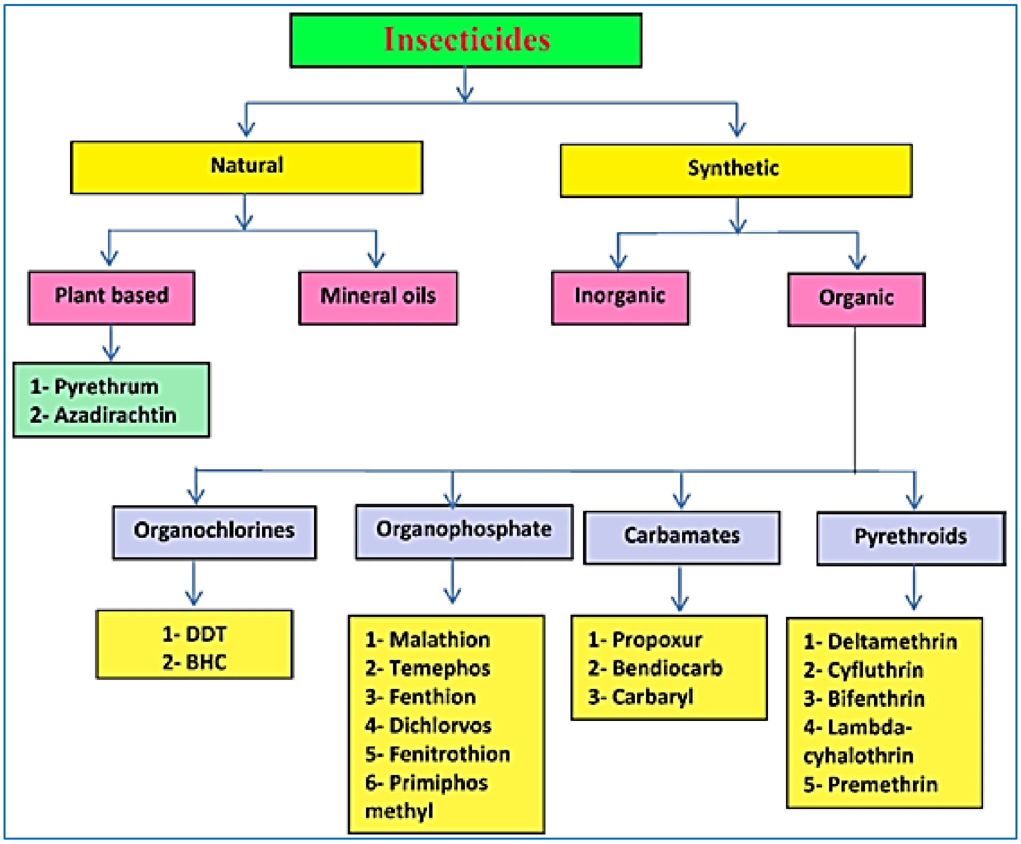
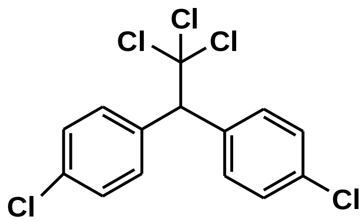


Figure 3: Classification of insecticides

Organochlorine

Organochlorine pesticides (also known as chlorinated hydrocarbons) are organic compounds with five or more chlorine atoms attached. Pesticides represent one of the first groups of pesticides ever synthesized and used in agriculture and public health. Most of these plants are widely used as insecticides for the control of a wide range of insects, and they have long-term residual effects on the environment. These insecticides may disrupt the nervous system of insects, leading to convulsions and paralysis followed by eventual death. The most common examples of these pesticides include DDT, lindane, endosulfan, aldrin, dieldrin and chlordane. Although the production and application of DDT were banned in most developed countries, including the United States, many years ago, DDT has still been used in most tropical developing countries for vector control (particularly where malaria occurs). Structural examples of some Organochlorine pesticides are shown in Figure 4 below.



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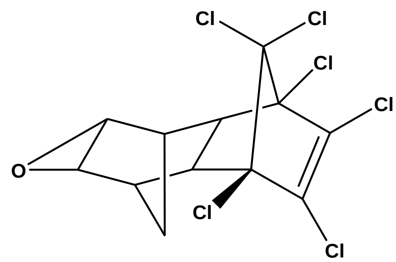

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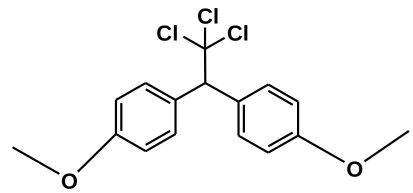

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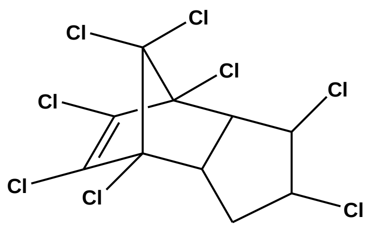

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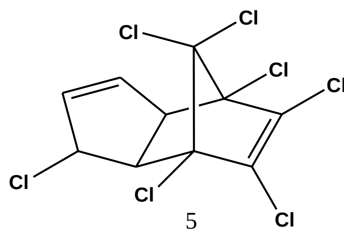

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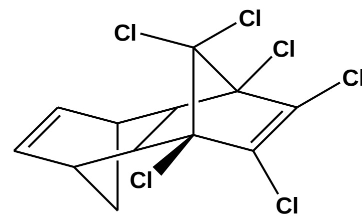

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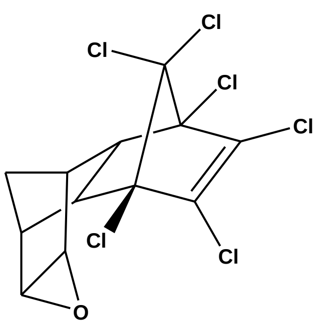

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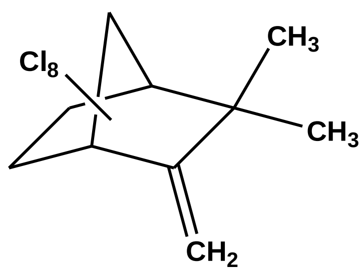

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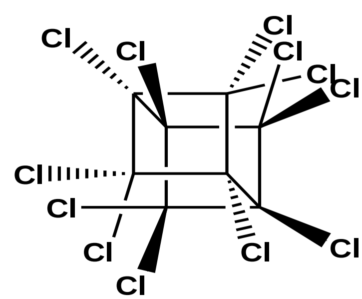

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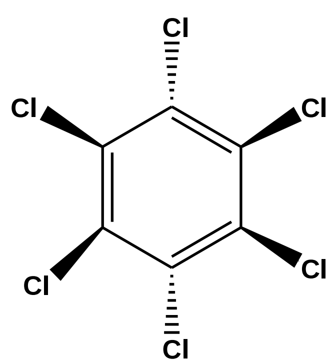

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Figure 4: Examples of organochlorine insecticide structures

Organophosphates

Organophosphate pesticides are considered to be broad-spectrum pesticides that control a wide range of pests due to their multiple functions. These infections are characterized by stomach poisoning, contact poisoning and fumigant poisoning, all of which can lead to nerve poisoning. These pesticides are also biodegradable and cause minimal environmental pollution and slow pest resistance.[100] Organ phosphorus insecticides, as cholinesterase inhibitors, are more toxic to vertebrates and invertebrates than are other insecticides, leading to a permanent overlay of acetylcholine neurotransmitters across a synapse. As a result, nervous impulses fail to move across the synapse, causing rapid twitching of voluntary muscles, hence leading to paralysis and death. Some of the widely used organophosphorus insecticides include parathion, malathion, diaznon and glyphosate.

Carbamates

Structurally, carbamates are similar to organophosphates. However, they differ in their origin. Organophosphates are derivatives of phosphoric acid, while carbamates are derived from carbamic acid. The working principle of carbamate pesticides is similar to that of organophosphate pesticides; carbamate pesticides affect the transmission of nerve signals, resulting in the death of the pest by poisoning [101]. Sometimes, they are also used as stomachs and contact poisons as well as fumigants. Heavy metals can be easily degraded in a natural environment with minimal environmental pollution. Some of the widely used insecticides in this group include carbaryl, carbofuran, propoxur and aminocarb. Structure of one of carbamate pesticides is shown in Figure 5 below.

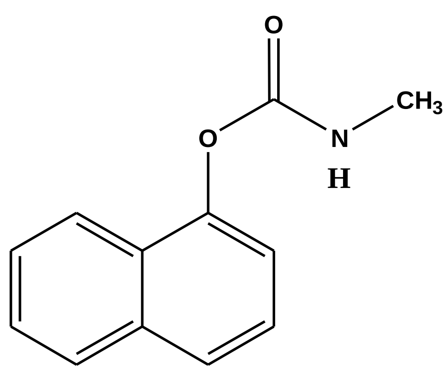

Figure 5: Example of the structure of the carbamate insecticide

2.2.3 The fate of pesticides in the environment

Pesticides are distributed into four major compartments after they are applied in the field, water, air, soil, or biota (living organisms).[102] The amount of pesticide that moves into each compartment depends on the physicochemical properties of the pesticide. Physical processes, such as sedimentation, adsorption, and volatilization, play vital roles in the distribution of pesticides in the environment.[102,103] Following this, they can be degraded by chemical and biological processes. The physicochemical characteristics of the pesticide (water solubility, absorption into 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.[102] Generally, solubility, hydrolysis, volatility, photodegradation, microbial degradation, leaching, and oxidation/reduction are factors that determine the fate of pesticides in the environment.[102–105] In addition, environmental weather conditions play a greater role in determining the fate of pesticides. The specific pesticide has different dissipation rates in tropical and temperate regions. The degradation rate of pesticides in soils from temperate and tropical regions has been investigated under controlled laboratory conditions, and the results revealed no difference in degradation rate.[103,104] However, field investigations have shown that a greater degradation rate occurs in tropical regions. This shows that pesticide degradation is dependent on environmental weather conditions.[104,105] Distribution of pesticides throughout environmental compartments is shown in Figure 6.

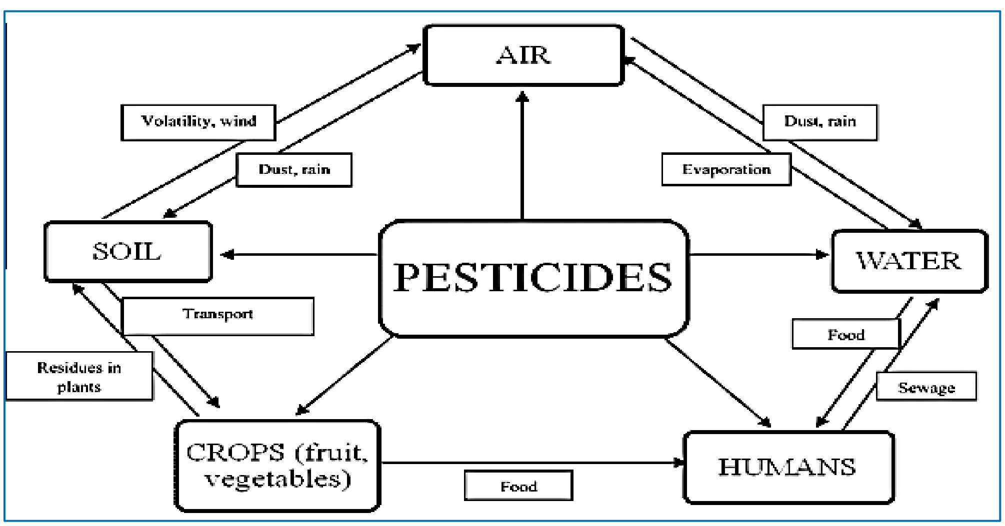

Figure 6: Circulation of pesticides in nature, including crops[106]

2.2.4 Exploring the presence of pesticide residue in Khat: A review of past research in Ethiopia

The work of Mekonen et al.[28] on exposure to DDT and its metabolites from Khat (Catha edulis) chewing: Consumers risk assessment from southwestern Ethiopia has shown the results of DDT and its metabolites detected in Khat samples. Based on these results, p,p- DDE, p,p’-DDD, p,p’-DDT and o,p’-DDT were detected in 80%, 70%, 61.7% and 58.3%, respectively, of the Khat samples. Total DDT, which is the sum of DDT metabolites and isomers, was detected in 80% of the Khat samples. The primary metabolites p,p’-DDE and p,p’-DDD were detected in a large number of Khat samples. The mean total DDT residue in Khat was generally greater in samples from the Sokoru (71.70 mg/kg), Dedo (149.00 mg/kg), Saka (137.00 mg/kg), Kersa (103.00 mg/kg) and Mana (73.00 mg/kg) districts, except for the samples from the Gomma district, which were relatively low (41.20 mg/kg) compared to those from the other five districts.

Another study conducted by Abdulaziz[107] investigated the presence and level of pesticide residues in Khat samples collected from various parts of Ethiopia. Khat samples had p,p’-DDT concentrations ranging from 141.2 to 973.0 µg/kg. Maximum concentrations were found between 240 and 1200 times the European Union maximum recommended levels for DDT in food (10 µg/kg vegetables and 50 µg/kg cereals). In a recent study on DDT, the concentrations were 11.3 µg/kg for p,p’-DDT and 26 µg/kg for the metabolite p’p-DDE. A lower concentration of p’p-DDE was associated with decreased application of p,p’-DDE during Khat cultivation.

A study conducted in the Sidama zone in southern Ethiopia by Ligani and Hussen[108] revealed that p,p’-DDT and p,p’-DDE were detected in all the samples. Similarly, the compound p,p’-DDD was detected in samples from five of the seven districts. Lakebay and Shebedino were the exceptions. The concentrations of p,p’-DDT, p,p’-DDE, p,p’-DDD investigated varied from 10.8 to 19.7, 3.5 to 18.6, and 2.0 to 6.5 µg/kg, respectively. Similarly, the p,p’-DDT, p,p’-DDE, p,p’-DDD concentrations in the Chuco district were 19.7 ± 2.1, 18.6 ± 1.3 and 6.5 ± 0.8 µg/kg, respectively. The mean total DDT concentration determined in this study did not exceed the recent maximum residue limit (MRL) recommended by the FAO/WHO (100–200 µg/kg) for different agricultural food items[109] or exceeded the European Commission (EC) MRL value of 50 µg/kg[17] and Japanese MRL value of 200 µg/kg for different food items.[110]

2.3 Exploring the Potential of Khat Leftovers as a Sustainable and Economical Feed Source for Goats and Sheep in Ethiopia

Numerous research findings have highlighted the value of Khat as a source of animal feed.[23,24] Khat (edulis) leftovers are one such source of animal feed in Ethiopia. The vast majority of smallholder farmers in Eastern and Southern Ethiopia cultivate Khat, a perennial income crop.[111] Despite the fact that millions of people use and consume Khat daily and that Khat leftovers are fed to livestock in Ethiopia and other nations, very little is known about Khat acceptability and nutritional components for feeding animals as the only diet or as a supplement. Fresh Khat leftovers are regarded as nontraditional feeds with respectable nutritional value; these feeds include better gross energy and calcium, a moderate amount of CP, and in vitro-organic matter digestibility (IVOMD). However, adequate information on the pesticide contamination level in Khat leftover, which is used as a nonconventional feed in the diets of goats and sheep, has not been exhaustively studied and documented, except for a few studies relying on the use of Khat (*edulis*) leftover meal as feed for sheep and its implications for feed intake, digestibility and growth.[112] Information on the effectiveness of residual Khat in goat feed[113,114] has demonstrated its usefulness in improving goat nutrition. Therefore, this study was designed to evaluate the contamination level of OCP residues in Khat leftovers used as nonconventional feed in goat and sheep feed.

2.4 Sample preparation for pesticide determination

Sample preparation is the first step in any instrument analysis, including separation or extraction of the desired analytes from the sample matrix, as they are present in trace amounts (typically μg/kg or less). It eliminates any interference and reduces the volume of the extract, thereby concentrating the analyte.[115] The type, nature, composition of the sample and concentration of the analyte to be separated or extracted determine the choice of separation and detection method to be used. This also indicates how sample preparation will be used. The efficiency of any analysis is determined by the sample preparation step.[116] On the other hand, in line with current trends, analytical procedures should aim to minimize and simplify the sample preparation step while maintaining high productivity, low operating costs, and process improvement. Sample preparation included extraction, concentration, separation, and analyte cleaning. This effort focuses on sample preparation, where we move from traditional labor-intensive methods to new methods that are quick and simple, such as the quick, easy, inexpensive, efficient, robust, and safe multiresidue method (QuEChERS).[115–118]

2.4.1. Concept and use of the QuEChERS technique

Considerable effort has been made in the past two decades to adapt existing sample preparation methods and develop new methods that save time, labor, and materials.[119] It is estimated that the sample preparation step in most determinations consumes approximately 60–70% of the total time required for the analysis.[117] QuEChERS (fast, easy, cheap, effective, robust, and safe) was first introduced for the analysis of pesticide residues in fruits and vegetables with high water content and has become a common method of preparation worldwide.[108,120] Recently, however, this method has gained popularity for the analysis of pesticides and other compounds present in a variety of food products and different matrices.[121,122] The QuEChERS method and its modifications are now rapidly expanding beyond their original scope to analyze multiple residues in different matrices. Polar and nonpolar compounds are extracted simultaneously, with the initial extraction involving the use of organic solvents, followed by separation by the addition of a salt mixture and final cleaning.

Currently, dispersed solid-phase extraction (d-SPE) is the most widely used cleaning method. The dSPE method is similar to SPE, but the solid phase, such as C18, PSA, or GCB, is added directly and facilitates the cleaning process. This cleaning process is widely used after performing QuEChERS extraction in multiresidue analysis.[123] The use of absorbent PSA is standardized, as is the addition of magnesium sulfate to remove undesirable substances (sugars, fatty acids, and water) from organic solvents in GC applications. The use of salts such as magnesium sulfate to induce large exothermic separations of pesticides from aqueous to organic phases is highly important in this process.[124–126]

2.5 Instrument used for pesticide residue determination

Various instrumental techniques are used to determine pesticide residues in foods. The most commonly used methods are gas chromatography (GC), gas chromatography coupled with mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), and liquid chromatography coupled with mass spectrometry (LC–MS).

Gas chromatography–mass spectrometry (GC–MS)

In GC–MS, pesticide concentrations were determined by retention time, and specific ions were determined using selective ion monitoring (SIM) mode with targeted and qualified ions. The SIM provides adequate low-level quantification for monitoring purposes, but the reliability of identity confirmation is reduced if the selected ions are affected by matrix effects. In addition to the use of MS/MS, it is possible to reduce the background effect and achieve a higher degree of selectivity and a lower limit of detection.[127] GC–MS/MS with three quadrupoles[128] and an ion trap mass spectrometer[129] were used to analyze pesticide residues on fatty foods. Acquisition, multiple-reaction monitoring, and selective response monitoring (SRM) methods[128] have been used to analyze several pesticide residues from food samples. The use of MS/MS can solve problems related to chromatographic interference that occur with GC-ECD.[128] Several single- and multiple-residue methods using GC–MS have been developed for the analysis of pesticides of different origins.[130–132]

3. MATERIALS AND METHODS

3.1 Study site

The present study was conducted in Jimma City, southwestern Ethiopia. Jimma Zone is one of the khat-growing areas in Ethiopia. The study site includes four districts (Kersa, Seka, Dedo, and Mana) and one kebele (Jiren) of Jimma Zone, which are considered sources of chewable Khat for the local market in Jimma City. Additionally, the leftover Khat sample was collected from a dominantly khat-producing district, namely Kersa, Seka and Mana districts. A map of the study site and the sampling location are given in Figure 7.

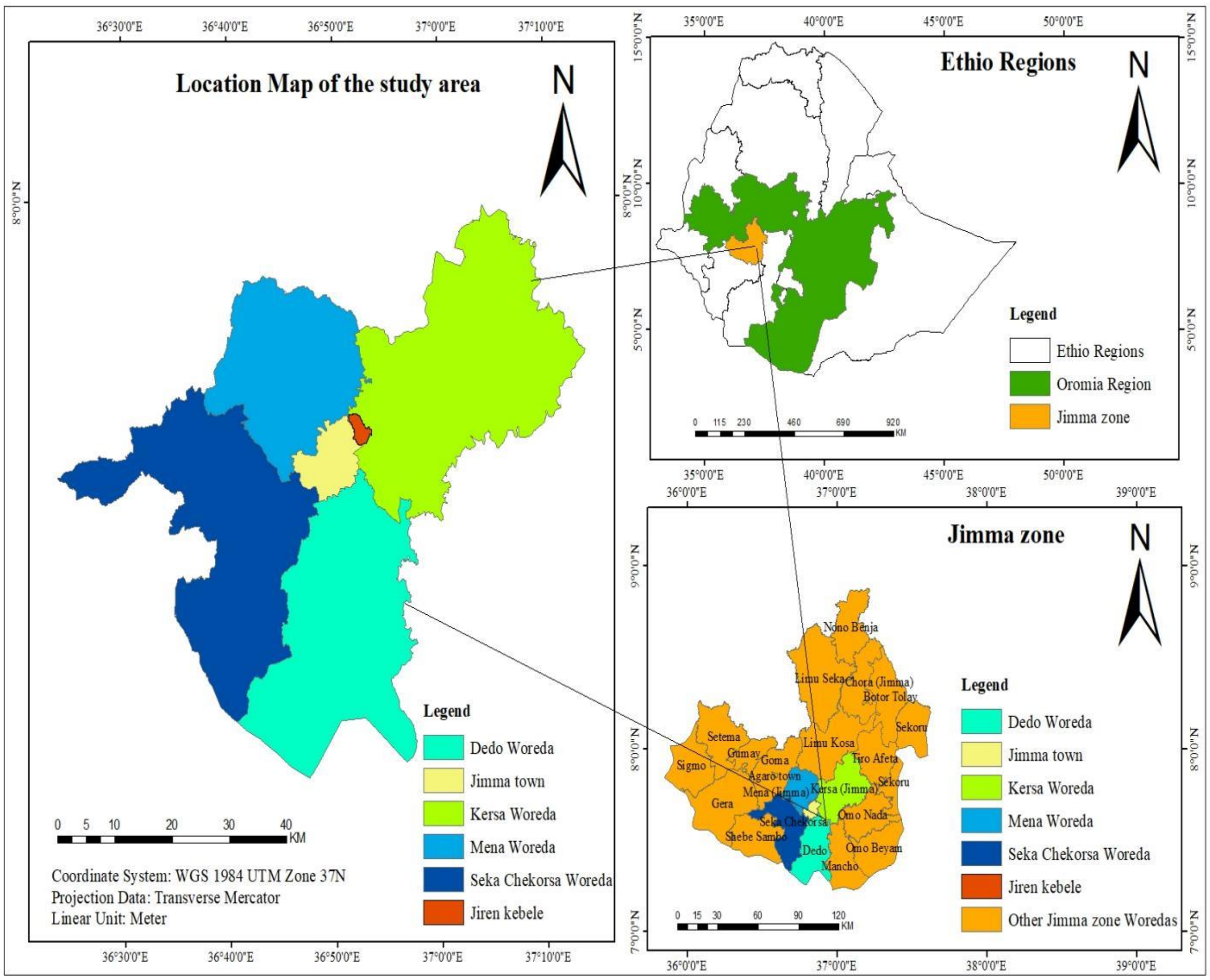

Figure 7: Map of the study site, including the sample source

3.2 Study design

An experimental study was conducted to determine the organochlorine pesticide residue in chewable Khat samples purchased from major market centers in Jimma city. Kersa, Seka, Dedo, and Mana) and one kebele (Jiren) of Jimma Zone were considered as a sources of chewable Khat samples for the local market, and leftover Khat samples collected from the three predominant Khat producing and supplying districts, namely, Mana, Kersa and Seka, in the Jimma zone, southwestern Ethiopia.

3.3 Sampling

Khat sampling is a meticulous process that involves the careful selection and analysis of both chewable and leftover Khat leaves. The samples were selected purposively from different Khat markets in Jimma city and three major Khat-producing districts in the Jimma zone. The samples were purchased in June 2023. Prior to the purchase of the samples, the markets were assessed for the suitability of proper sampling, and the Khat sellers from each market were orally interviewed on the basis of the origin of their Khat, and Khat cultivators were again orally interviewed on the leftover (mature leaves) Khat remaining from the processes of preparing chewable Khat for sell. Additionally, Khat consumers were also orally interviewed on the type of Khat most commonly consumed. In this particular study, a total of 120 leaves with tender stems were sampled, 60 of which were tender steam (chewable) Khat leaves that were purchased from the bustling markets of Jimma city (Mercator, Ajip, Koche, Central, Sekaber, and Areboch tera Khat markets). The four districts (Kersa, Seka, Dedo, and Mana) and one kebele (Jiren) are considered as a source of chewable Khat for local markets of Jimma city. The remaining 60 leaves were obtained from mature (leftover) Khat samples collected from three major districts, namely, Mana, Seka, and Kersa. In every market, fresh Khat samples were purchased from different sellers to ensure accurate representation. After collection, the samples were sealed in polyethylene bags, labeled appropriately and transported to the laboratory. The samples were further stratified by their sources into two portions, namely, chewable or tender Khat and the leftover parts. Then, approximately 300 g to 350 g of chewable parts were taken from each Khat sample as a laboratory sample and kept in a refrigerator for further analysis. In addition to the chewable parts of the Khat samples, the leftover Khat samples collected from the three major districts were mixed to represent the composite of the leftover Khat samples and were again kept in a refrigerator for further analysis.

3.4 Chemicals and Materials

3.4.1 Chemicals

i. Pesticide standards

Analytical grade OCP standards, namely, benzene hexachloride (BHC) (α-BHC, 99.5%; β-BHC, 99.5%; and δ-BHC, 99.5%); gamma-chlordane (γ-Chlor) (98.8%); p,p'-DDE (99.99%); p,p'-DDT (98.9%); and methoxychlor (MC) (97.7%), were purchased from Sigma Aldrich (St. Louis, MO, USA).

Standard OCP solution preparation

Individual stock standard solutions of 400 mg/L DDE, DDT, gamma chlordane, and MC; 1000 mg/L β-BHC, δ-BHC, and α-BHC; and 800 mg/L aldrin were prepared in hexane. A mixed intermediate standard solution containing 20 mg/L of each OCP was prepared in hexane from the stock solutions. All the prepared standard solutions were stored at 4°C in a refrigerator until they were used for analysis. Working standard solutions were prepared daily by diluting the mixed intermediate standard solution in hexane, after which 5 µg/L, 10 µg/L, 20 µg/L, 30 µg/L, 40 µg/L, and 50 µg/L standard solutions were prepared. These working standard concentrations were injected into the GC–MS system to create a chromatogram of the peaks that could be used to construct a standard calibration curve and establish LODs and LOQs. The standard was completely prepared at ambient temperature, and the solutions were kept in a deep freezer until needed. The working standard solution and the sample extract were removed from the deep freezer and allowed to thaw at room temperature before being injected into the GC during analysis.

ii. Organic solvents and reagents

Analytical grade solvents, including acetonitrile and hexane, were obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). Anhydrous magnesium sulfate ($MgSO_4$) and sodium chloride (NaCl) of analytical grade were obtained from Sigma–Aldrich (Steinheim, Germany). Other necessary materials were Florisil adsorbent (60–100 mesh, Merck, residue grade), which was obtained from BDH chemical Ltd. (Poole England).

3.4.2 Materials

This work was conducted using a variety of instruments and equipment, including an Agilent 8890 GC coupled with an Agilent 5977B single quadruple MSD and an Agilent G4513A autosampler

(Agilent Technologies, USA), which were used for the analysis of OCPs. An HP-5MS capillary column (30 m, 0.25 mm id., 0.25 μm film thickness) coated with 5% diphenyl 95% dimethylsiloxane obtained from Agilent Technologies was used for analyte separation; centrifuge(s) (capable of holding 50 mL and 15 mL centrifuge tubes used for extraction: model KARL KOLB D- 6072 (Germany and PLC02, Taiwan, respectively); a freezer (capable of continuous operation < 4°C); an oven for material drying; a rotary evaporator: model LABOROTA 4000 (Buchi, Switzerland); a vortex mixer from Fisher Scientific (model FB15024, Belgium); and an Agilent Technologies 2 mL amber cup vial. Additional apparatuses, including Falcon plastic centrifuge tubes with 15 and 50 mL capacities and micropipettes of various sizes, were used in the process of sample preparation.

3.5 Sample preparation

For the analysis of OCPs in the Khat samples, we adopted an extraction and clean-up method with slight modifications from Winnette A et al.[133], a newly modified QuEChERS method for the analysis of organochlorine and organophosphate pesticide residues in fruits and vegetables.

3.5.1 Sample Extraction and Clean-up

The samples were initially screened to determine if pesticide residues were present. Once no pesticide was detected, the plants were considered blank extracts. The Khat samples were homogenized using a mortar and pestle, and 10 g of the homogenate was placed into 50 mL centrifuge tubes. Fifteen milliliters of acetonitrile was added to each centrifuge tube, and the samples were vortexed for 1 min. Four grams of anhydrous magnesium sulfate and 1 g of sodium chloride were subsequently added to the tubes. The samples were subsequently centrifuged for 5 min at 5000 rpm. Seven milliliters of the supernatant was transferred to 15 mL dispersive tubes containing 0.250 g of Florisil sorbent and 0.750 g of anhydrous $MgSO_4$. The analytical procedure is explained in Figure 8.

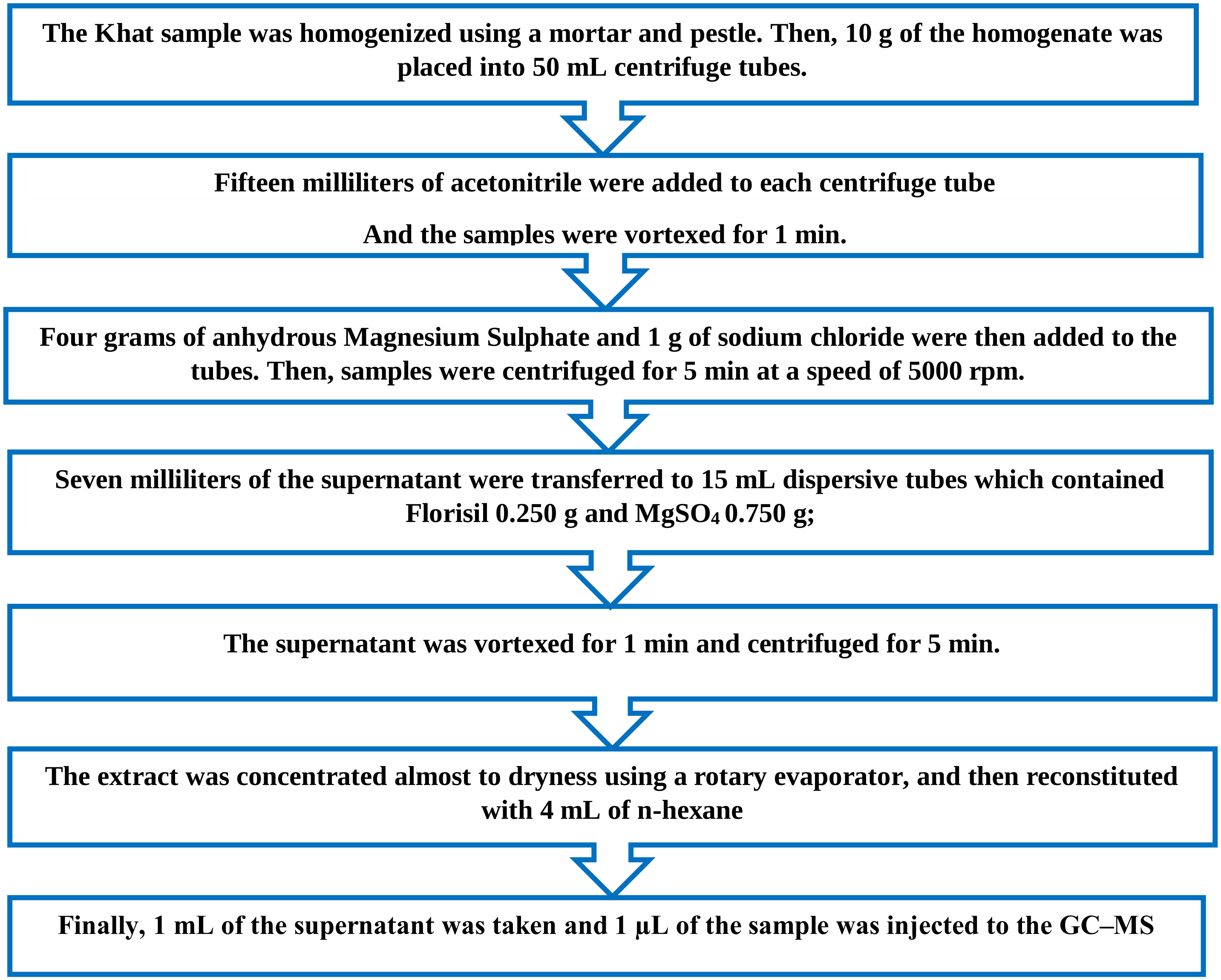

Figure 8: Flowchart of the extraction and clean-up procedure

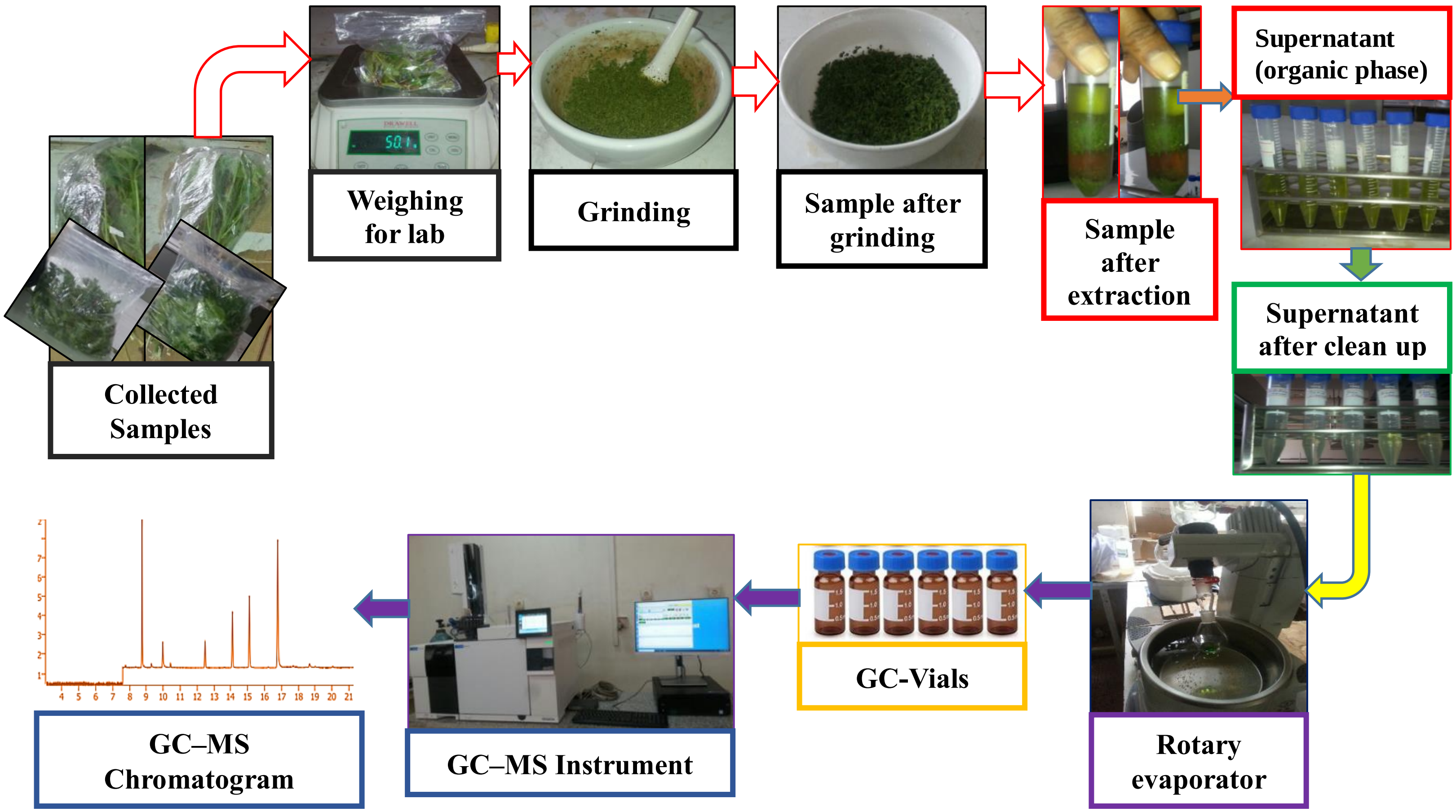

Figure 9: Flowchart of extraction and clean-up GC–MS analysis

3.5.2 GC–MS operating conditions

High purity helium was used as the carrier gas at a flow rate of 1 mL/min. In a split-less injection, 1 L of the sample was used. The injection port temperature was changed to 280°C. The GC oven temperature program consisted of an initial temperature of 100°C, a ramp at 15°C/min to 200°C (for 5 min), a ramp at 4°C/min to 250°C (for 2 min), and a ramp at 10°C/min to 270°C (for 10 min). The GC–MSD transfer line, MSD ion source, and quadrupole had respective temperatures of 280, 230, and 150°C. The MSD was run in electron ionization mode with a 70 eV ionization energy, scanning at 150 scans/second from m/z 45 to 500, and a 3 min solvent delay time. Using one quantitative and two qualifier ions, the OCPs were determined in the SIM (selected ion monitoring) mode.

3.6 Method validation

3.6.1 Linearity of the standard curves

The linearity of all the organochlorine pesticides was demonstrated. For all of the pesticides of interest, linear spiked calibration curves were established with correlation coefficients that varied between 0.995 and 0.999.

3.6.2 Limits of detection and limits of quantification

The limits of detection (LODs) and limits of quantification (LOQs) of the methods were computed by taking into account 3 and 10 times the background noise, respectively, and evaluated via spiked serial dilutions of working standards generated for calibration curves.

$$\mathrm{LOD} = 3\mathrm{S/N}\ \mathrm{LOQ} = 10\mathrm{S/N}$$

3.6.3 Recovery studies

Loss of target compounds can occur during sample preparation, extraction and analysis. The extent of analyte loss, especially during extraction, was assessed by performing a recovery test. This was done by spiking the samples with 10 µg/kg of an internal standard before extraction to evaluate the recovery of the compounds. The recovery was determined using the following formula:

$$\%\ \mathrm{Recovery} = \frac{\mathrm{Pesticide\ (ppb) recovered\ from\ fortified\ Khat\ sample}}{\mathrm{Amount\ of\ pesticide\ (\mu g/kg)\ added}}\ \mathrm{x}\ 100$$

3.7 Data analysis

The statistical analysis included the mean of the samples and the corresponding standard deviations. Ranges were compiled from minimum and maximum values for pesticide residue levels detected in the study. The data were subjected to one-way analysis of variance to determine the differences in pesticide residue among the samples as well as the different areas from which they were sampled. All these calculations were performed using Microsoft Excel 2016. All the analysis reports were generated by Mass-Hunter GC–MS software.

4. RESULTS AND DISCUSSION

The aim of this study was to measure the amount of selected organochlorine pesticide residue in chewable and leftover Khat samples. Eight target organochlorine pesticides were the focus of the analyses of Khat samples that were available on the market. The analysis method was validated prior to performing the analyses, as shown in the next subsection.

4.1 Method Validation

The results corresponding to the percentage recoveries, regression coefficients, and precisions in terms of relative standard deviation (RSD) are summarized in Table 1.

Table 1: Method validation results for Khat residue analysis: regression coefficient, recovery %, linear dynamic range, limit of detection (LOD), and limit of quantification (LOQ) (μg/kg).

Pesticides	R^2	Recovery (%)	LDR (μg/kg)	LOD (μg/kg)	LOQ (μg/kg)
α-BHC	0.996	83.2	16-50	4.8	16
β-BHC	0.996	86.3	12-50	3.6	12
δ-BHC	0.997	81.2	6-50	1.8	6
Aldrin	0.997	90.4	16-50	4.8	16
Methox-chlor	0.997	93.3	8-50	2.4	8
γ-Chlordane	0.997	103.3	7-50	2.2	7
p, p'-DDE	0.997	101.8	14-50	4.2	14
p, p'-DDT	0.995	82	10.3-50	3.1	10.3

According to European Commission regulations, the percent recovery should be between 70% and 120%.[134] In this study, the percentage recoveries of the organochlorine pesticides were found to be acceptable, ranging from 81.2% for δ-BHC to 103.3% for γ-Chlordane (Table 1), which indicates that the method is, therefore, applicable for the reasonable determination of eight OC pesticides in both chewable and leftover Khat samples. The limits of detection (LODs) and limits of quantification (LOQs) varied from 1.8-4.8 μg/kg and from 6-16 μg/kg, respectively. Therefore, the results showed that the applied method was reliable for the analysis of pesticide residues in this study.

The calibration curves were obtained using analytical solutions of the mixture of the pesticides prepared in pure solvent and prepared from the extract of the matrix in the concentration range.[135] The acceptability of linear data is often judged by examining the correlation coefficient and the y intercept of the linear regression line for the response versus concentration. A correlation coefficient that varies between 0.995 and 0.999 is generally considered acceptable. The y intercept should be less than a few percent of the response obtained from the target level.[136]

Accordingly, calibration curves were obtained from running six-point calibration solutions with concentrations ranging from 5 µg/kg to 50 µg/kg. The lowest concentration in the calibration curve was established as the practical determination limit for the instrument. Linearity was evaluated by calculating six-point linear plots of the peak height (as observed Figure 10*:* A representative chromatogram of 50 µg/kg standard analyzed for 1. α-HCH; 2. B-HCH; 3. δ-HCH; 4. Aldrin; 5. Methox-chlor; 6. γ for a representative chromatogram from the six-concentration range) against the concentration based on linear regression and the squared correlation coefficient, r^2, which should be > 0.99. As shown above (Table 1), all of the pesticides had good linearity and correlation coefficients (r^2) >0.99. The calibration curves for several pesticide standards are presented in Figure 14**:** . (Refer to Appendix B: Calibration graph with for the remaining six calibrations.) In addition to this Figure 11 - 13 shows chromatogram of Khat sample free of pesticide, Khat sample contaminated with pesticides and Khat sample spiked at 10 µg/kg respectively.

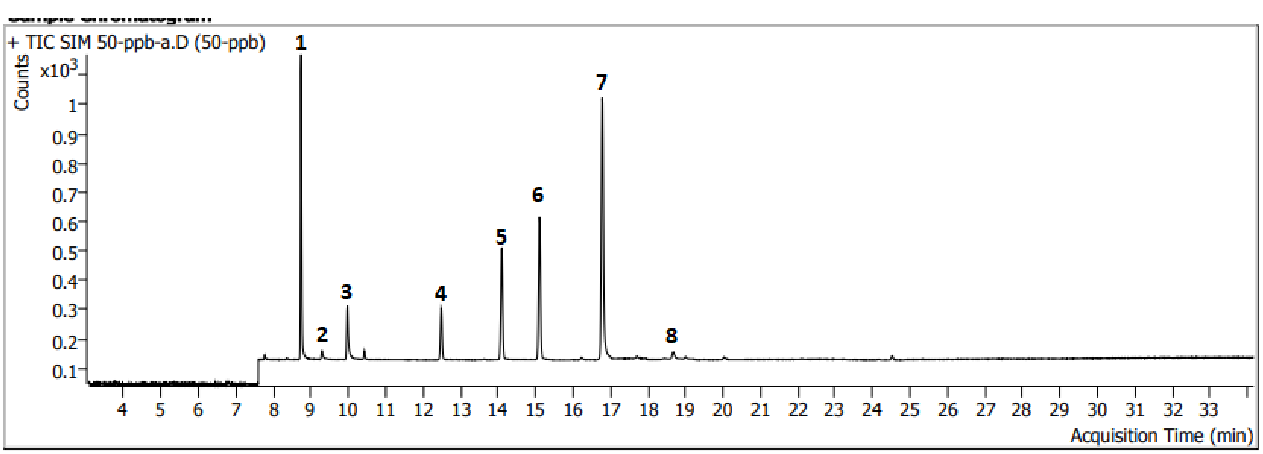

Figure 10: A representative chromatogram of 50 µg/kg standard analyzed for 1. α-HCH; 2. B-HCH; 3. δ-HCH; 4. Aldrin; 5. Methox-chlor; 6. γ-Chlordane; 7. DDE; 8. DDT (Refer to Appendix C: *Chromatogram of the studied pesticides*.

4.1.1 Repeatability and Reproducibility

The repeatability of the method (intraday) was studied by running six (6) extractions, which were spiked with a 10 μg/kg OCP mixture within one day of extraction Figure 12:The %RSD values of all the pesticides were less than 20%, as shown in (Table 2). However, the reproducibility of the method (interday) was investigated by running six (6) spiked samples on different days of extraction. The percentage RSD was also less than 20%. This indicates that the method is reasonably repeatable and reproducible under the laboratory conditions available in the program.[137,138]

Table 2: Repeatability and reproducibility of the studied pesticides in fortified extracts of chewable and leftover Khat samples extracted by the newly modified QuEChERS method [133] and analyzed using GC–MS

	Intraday %RSD, n = 6		**Interday %RSD, n = 6**	
Compounds	**Chewable Khat**	**Leftover Khat**	**Chewable Khat**	**Leftover Khat**
α-BHC	8.987	7.722	8.568	7.132
β-BHC	7.837	9.142	4.036	8.717
δ-BHC	6.748	4.058	2.809	4.104
Aldrin	9.015	8.785	7.264	9.904
M-chlor	8.695	1.589	8.291	5.648
γ-chlor	5.376	2.140	5.126	9.326
p, p’-DDE	1.027	2.716	3.455	7.450
p, p’-DDT	6.182	1.018	6.691	4.506

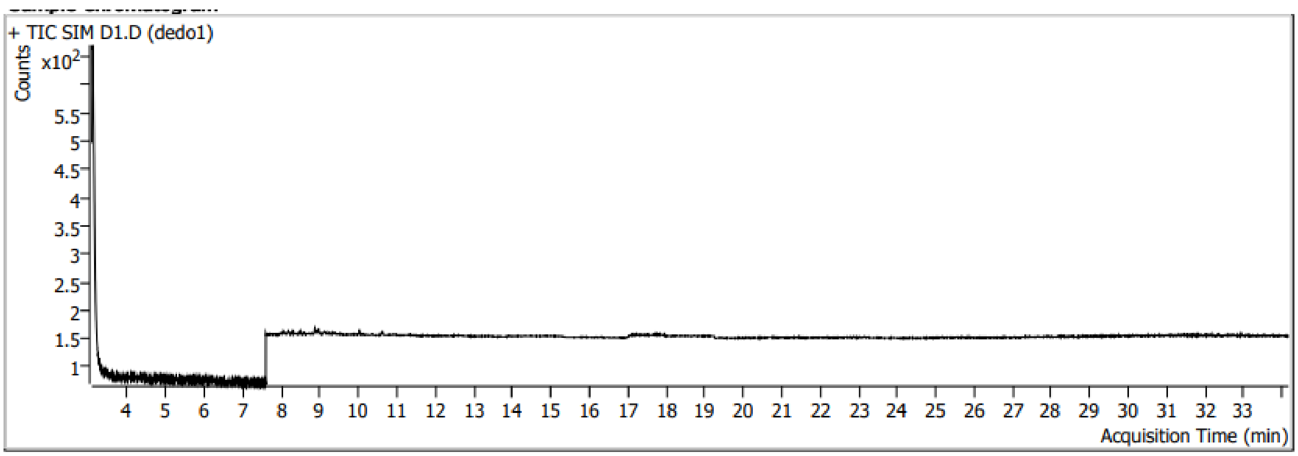

Figure 11: A representative chromatogram of a Khat sample free of pesticide

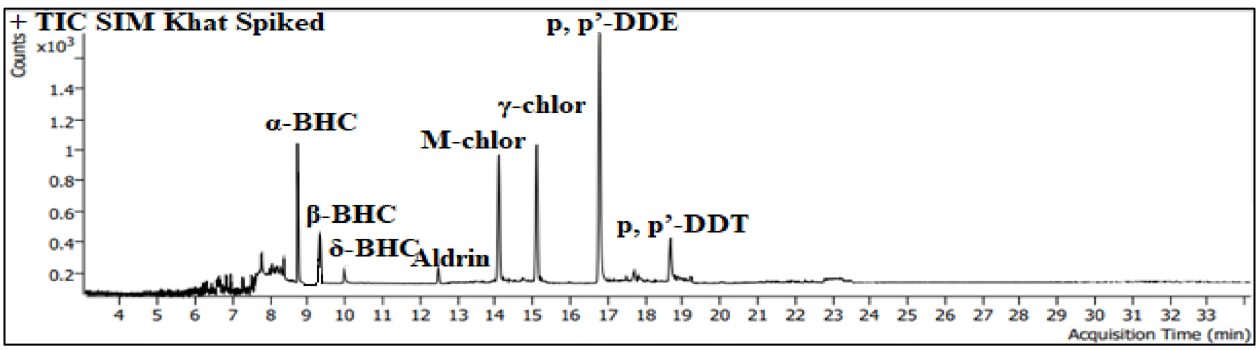

Figure 12: GC–MS chromatogram of a Khat sample spiked at 10 µg/kg

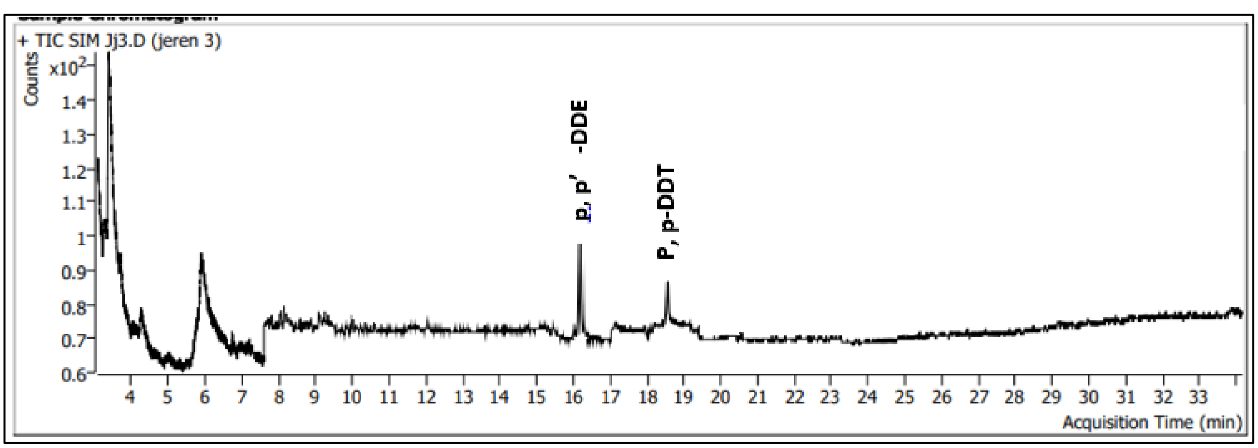

Figure 13: GC–MS chromatogram of the Khat sample contaminated with pesticides

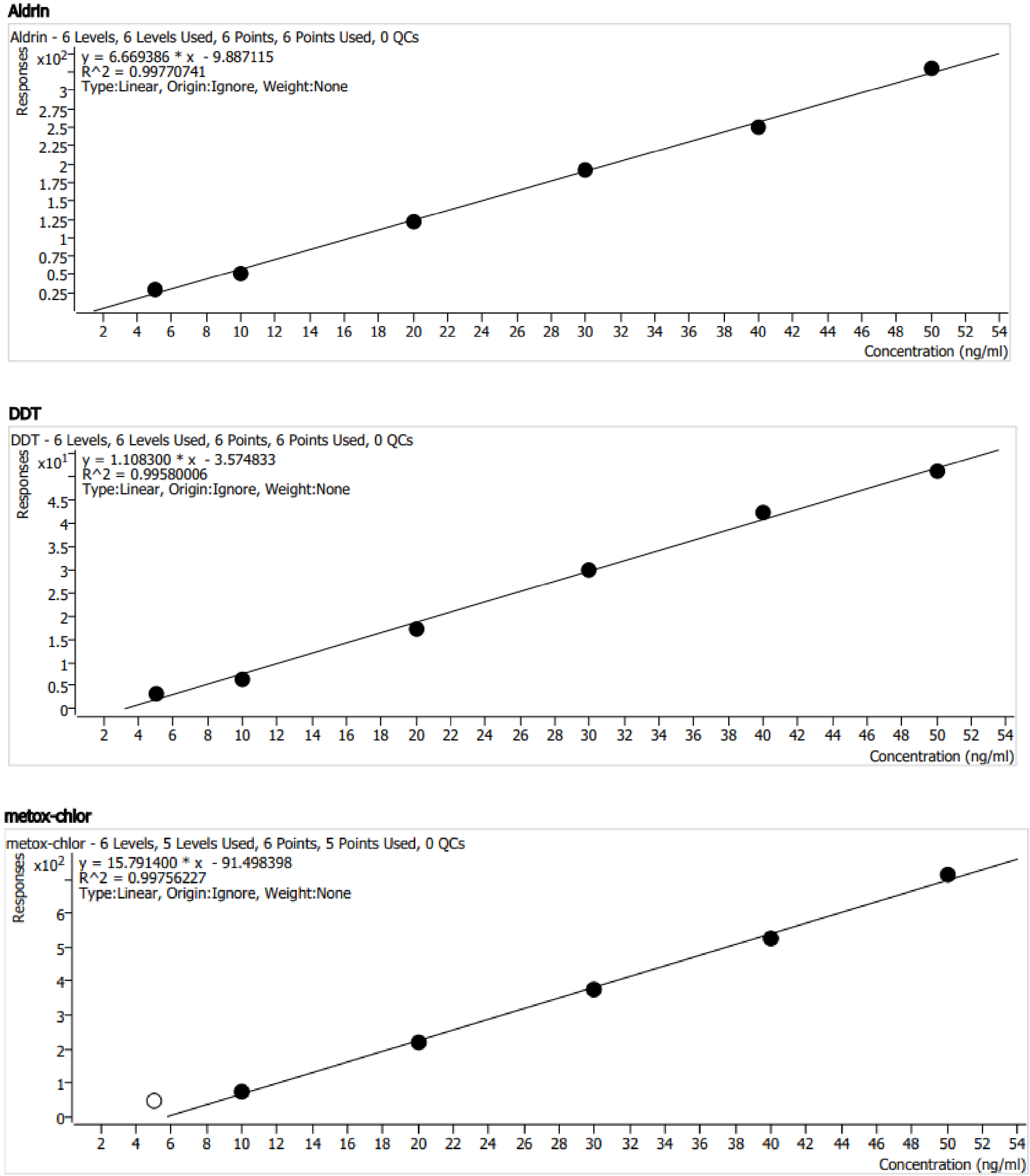

Figure 14: Calibration curves for aldrin, DDT, and methox-chlor

4.2 Sample analysis

After the validation of the method, Khat samples were collected from Jimma city Khat sellers and analyzed for the 8 selected pesticide residues. (Refer to Appendix A: Files during sample collection and extraction timeBefore sampling Khat sellers and chewers were interviewed orally on the origin and types Khat predominantly available in Jimma City markets. Additionally, Khat cultivators were also interviewed orally to determine the type of pesticides that they are currently using. According to the responses of the most interviewees, five main sources of Khat are coming to this market: Mana districts, Kersa districts, Dedo districts, Seka districts and the Jiren kebele around Jimma city. According to the responses of other Khat farmers, they use pesticides based on availability and affordability. Additionally, the leftover Khat samples were collected from a dominantly Khat-producing district, namely the Kersa, Seka and Mana districts. According to the responses of the Khat farmers use leftover khat as feed for animals such as goats and sheep. Moreover, they also use leftover Khat as a compost by dumping it on the farm. The samples were initially screened to determine if pesticide residues were present. Once no pesticide was detected, the sample was considered a blank extract (Figure 11). Based on the preliminary assessment, it is possible to suspect the presence of OCP residue in Khat, which is sold in Jimma city. Therefore, Khat samples were collected from markets located in five different locations, including markets located in the central part of the city.

4.2.1 Concentration of pesticide residue in chewable tender leaves of Khat (*Catha edulis*) samples

The results for the OCPs detected in the Khat samples are given in Table 4. Based on these results, pp'-DDT and its primary metabolite pp'-DDE were detected in all the chewable Khat samples. However, the mean values of p,p'-DDT and its metabolite p,p'-DDE were very low in Jiren chewable Khat sample compared with the remaining four Khat samples namely Kersa, Seka, Dedo, and Mana.

Table 3: Mean concentrations (μg/kg ± SD) of OCPs in chewable Khat samples

Sample		Pesticides concentration (μg/kg)							
		α-BHC	β-BHC	δ-BHC	Aldrin	p, p'-DDT	p, p'-DDE	Methox-chlor	γ-Chlordane
Dedo	**Mean**	ND	ND	ND	ND	24.14±0.05	35.03±0.05	ND	ND
Seka	**Mean**	ND	ND	ND	ND	12.49±0.54	40.15±0.00	ND	ND
Kersa (Beda Buna)	**Mean**	ND	ND	ND	ND	11.17±0.02	34.07±0.06	ND	ND
Jiren	**Mean**	ND	ND	ND	ND	8.53±0.36	10.12±0.01	ND	ND
Mana (Buture)	**Mean**	ND	ND	ND	ND	34.15±0.03	45.39±0.09	ND	ND

ND, not detected

As shown in Table 3, six pesticide residues, namely, Aldrin, α-BHC, β-BHC, δ-BHC, α-chlordane, γ-chlordane, and Methox-chlor, were not detected in the samples from any of the districts in the Jimma zone by GC/MS. However, p,p'-DDT and its metabolite p,p'-DDE were detected in all chewable Khat samples obtained from Dedo, Seka, Kersa (Beda Buna), Jiren and Mana (Buture) districts in the Jimma zone in southwestern Ethiopia. The average concentrations of p,p'-DDT were 10 µg/kg, 11 µg/kg, 14 µg/kg, and 12 µg/kg in the Dedo, Seka, Kersa (Beda Buna), and Mana (Buture) Khat samples, respectively. The average concentrations of the p,p'-DDT metabolite p,p'-DDE were 35 µg/kg in Dedo, 30 µg/kg in Seka, 12 µg/kg in Kersa (Beda Buna), and 15 µg/kg in Mana (Buture) Khat samples. The values of p,p'-DDT and its metabolite p,p'-DDE measured in Khat samples collected from various Khat market centers in Jimma city in the present study are very low. Compared with a study performed in the Jimma zone by Mekonen et al.[28], the mean residue concentrations of p,p'-DDT, and p,p'-DDE detected in all Khat samples in the present study were lower. This indicates that there has been no recent application of DDT on Khat Islands in the present study area (Jimma Zone). Additionally, interviews with farmers confirmed that they are not currently utilizing DDT.

As reported in the literature[139] the DDE/DDT or DDT/DDE ratio has been used to determine whether the pesticide residues detected were from past or recent pesticide use. In the present study, as shown in Table 3, the concentration of p,p'-DDT was lower than the concentration of its primary metabolite p,p'-DDE. This indicates that there was no recent application of DDT to Khat plants in the study area, which is similar to the findings of Mekonen et al[28]. A number of publications indicate an accumulation of p,p'-DDE and p,p'-DDD in environmental samples, suggesting that DDT may have been used in the past.[140–142] The parent DDT is transformed into p,p'-DDE and p,p'-DDD with time, whereas recent DDT application did not actually occur in that environment.

Notably, p,p'-DDE is found solely in the environment as a result of DDT breakdown or pollution and has the potential to contaminate soil, water, and other earth minerals with this metabolite [143] Hence, the contamination of Khat in the Jimma zone may most likely be due to the historical use of DDT for public health purposes, such as the control of disease vectors.

Table 3 shows that p,p'-DDT and its metabolite p,p'-DDE were low in Jiren Khat sample. The average concentrations of p,p'-DDT and its metabolite p,p'-DDE were 8.53 µg/kg and 10.12 µg/kg, respectively, in one of the Khat samples, namely, Jiren, which was obtained from the local market in Jimma. These results are an indication that there might be low use of pesticides in the area. This could also be due to the degradation of pesticides over time. The post hoc test results are presented below in the box-whisker plot Figure 15**:** . There was a significant difference in the distribution of the concentrations of p,p’-DDT and its metabolite p,p’-DDE in the Khat samples (P value < 0.05).

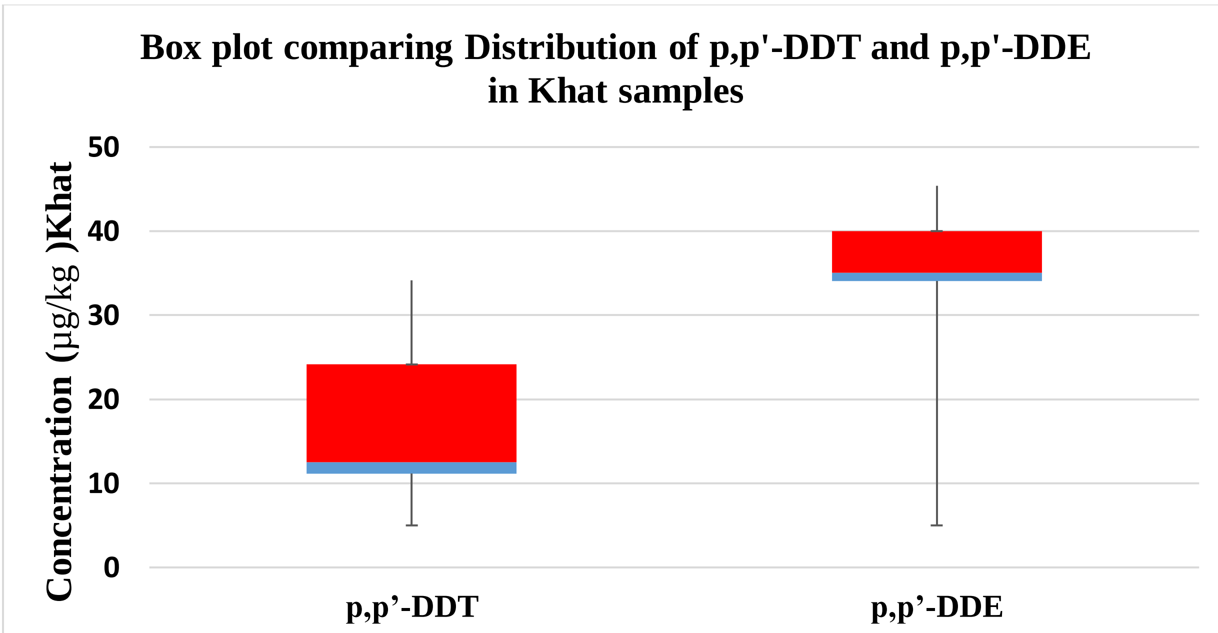

Figure 15: Distribution of the concentrations of p,p’-DDT and p,p’-DDT in Khat samples.

The p value corresponding to the F statistic of one-way ANOVA is greater than 0.05 (Table 4), suggesting that the five sample sources are not significantly different at that level of significance. (Refer to Appendix D: Analysis of Pesticide Concentration Variations Among the)The Tukey HSD test, as well as other multiple comparison tests like Scheffe or Bonferroni, might not narrow down which of the pairs of treatments are significantly different.

Table 4: Differences in the concentrations of total DDT between the Khat sample sources (the five districts of the Jimma zone)

Sample source Pair	**Tukey HSD Q statistic**	**Tukey HSD p value**	**Tukey HSD Inference**
A vs B	0.0778	0.8999947	Not significant
A vs C	0.3891	0.8999947	Not significant
A vs D	1.2709	0.8926328	Not significant
A vs E	0.4928	0.8999947	Not significant
B vs C	0.4669	0.8999947	Not significant
B vs D	1.3487	0.8623213	Not significant
B vs E	0.415	0.8999947	Not significant
C vs D	0.8818	0.8999947	Not significant
C vs E	0.8818	0.8999947	Not significant
D vs E	1.7637	0.7006457	Not significant

Tukey HSD comparison tests and chewable Khat sample sources, **A**: Dedo, **B**: Seka, **C**: Kersa (Beda Buna), **D**: Jiren, and **E**: Mana (Buture), were not significantly different (P value >0.05).

According to the Tukey HSD test, there was no significant difference in the concentrations of p,p'-DDT or its metabolite p,p'-DDE among the sources of the Khat sample, which included the five districts of Dedo, Seka, Kersa (Beda Buna), Jiren, and Mana (Buture), in the Jimma zone. Similar to the findings of Mekonen et al.[28] There was no significant variation in the concentration of total DDT among the Khat sample sources (P value > 0.700). This might be explained by the fact that all five districts may have had similar environmental factors, such as climate and soil type, which resulted in equal levels of DDT contamination in the Khat plants. The lack of noticeable changes in overall DDT concentrations may also be due to additional variables, including similar harvesting and processing practices across all districts.

4.2.2 Comparing findings: Current study vs. previous study in the same area

In a study conducted by Mekonen et al.[28] In the same study area, which is the Jimma zone of southwest Ethiopia, the concentrations of p,p'-DDT and its major metabolite p,p'-DDE in chewable sections of Khat leaves were examined. The researchers found that Khat samples from

Dedo, Mana, Seka, and Kersa contained p,p'-DDT and its principal metabolite, p,p'-DDE, at 25.97 and 113.28 µg/kg; 10.31 and 60.12 µg/kg; 18.83 and 64.13 µg/kg; and 68.94 and 16.71 µg/kg, respectively. However, in the present study, the concentrations of p,p'-1 DDT and its major metabolite p,p'-DDE found in the Khat samples were extremely low, with values of 13.14 and 35.03 µg/kg; 9.14 and 45.38 µg/kg; 12.48 and 40.14 µg/kg; and 11.16 and 34.06 µg/kg for Dedo, Mana, Seka, and Kersa, respectively. The higher concentration of the metabolite p,p'-DDE compared to that of the parent chemical p,p'-DDT indicates that DDT was previously used in southwestern Ethiopia.[28]

Several studies[141,142] have reported that high accumulations of p,p'-DDE and p,p'-DDD in environmental samples indicate that DDT could be historically used. The parent DDT is metabolized over time to p,p'-DDE and p,p'-DDD, while DDT has not been recently applied in these environments. Specifically, p,p'-DDE is found in the environment as a result of contamination or breakdown of DDT, which may contaminate soil and other earth materials with this metabolite.[144]

4.2.3 Comparison of the results obtained from the chewable Khat samples with the MRL

The average concentration of p,p'-DDT and its metabolite p,p'-DDE found in all chewable Khat samples in the present study (Table 3) was less than the most recent FAO/WHO maximum residue limit (MRL) of 100–200 µg/kg for various agricultural food items (FAO/WHO Food Standards Codex Almintarius, 2014) or an EC MRL value of 50 µg/kg and a Japanese MRL value of 200 µg/kg. However, the chewable Khat samples from Dedo, Saka, Kersa (Beda Buna), and Mana (Buture) contained p,p'-DDT and p,p'-DDE contaminants. It is challenging to determine whether the average concentration of p,p'-DDT and its metabolite p,p'-DDE is insufficient to cause concern to consumers despite being below the most current maximum residue limit (MRL). MRLs are not safe but rather indicate legal issues related to pesticide use, such as illegal use of obsolete or banned pesticides, the use of substandard formulations, or contamination from various sources, including those used to protect public health.[28] Therefore, additional research is needed to determine how these pesticides interact toxically and cumulatively with consumers.

4.3 Concentrations of selected organochlorine residues in Khat (*Catha edulis*) leftovers used for composting and animal feed

Eight OCP residue concentrations were measured in leftover samples of Khat taken from the Mana, Kersa, and Seka districts in the Jimma zone of southwest Ethiopia. The bulk of the pesticides investigated, including -BHC, -BHC, -BHC, alrin, methoxochlor, and -chordane, were not found in this study, as stated in Table. However, the only compounds found were p,p'-DDT and its metabolite, p,p'-DDE. In the leftover Khat samples, p,p'-DDT and its metabolite p,p'-DDE were the most common pollutants. Table 5 shows the average levels of p,p'-DDT and its metabolite p,p'-DDE in leftover Khat collected from the three districts. The mean values (µg/kg) for the Buture leftover Khat from the Mana District Farm were recorded as follows: p,p'-DDT (32.16) and p,p'-DDE (35.06). These numbers fell under the upper residue limit. A farm in Kersa district produced Beda Buna Khat with mean values (µg/kg) of p,p-DDE (30.12) and p,p'-DDT (6.73). However, the mean value of p,p'-DDT was below the quantification limit. The mean (µg/kg) concentrations of p,p'-DDT (22.12) and p,p'-DDE (45.04) were found in the leftover Seka Khat from a farm in the Seka district. Similarly to Khat samples from Kersa, the Seka Khat samples from Seka districts had p,p'-DDT and its metabolite p,p'-DDE levels below the maximum residue limits.

Table 5: Mean residual concentrations (µg/kg ± SD) of selected organochlorine pesticides in leftover Khat samples

LOK Sample name	Districts	α-BHC	β-BHC	δ-BHC	Aldrin	M-Chlor	γ-Chlor	p, p' – DDT	p, p' - DDE
Buture	**Mana**	ND	ND	ND	ND	ND	ND	32.16±0.06	35.06±0.07
Beda Buna	**Kersa**	ND	ND	ND	ND	ND	ND	6.73±0.01	30.12±0.06
Seka	**Seka**	ND	ND	ND	ND	ND	ND	22.21±0.04	45.04±0.09

ND: Not detected; **LOK**: Leftover Khat

These findings suggest that there may not be extensive pesticide use in the region. However, p,p'-DDT and its metabolite p,p'-DDE were present in leftover Khat, which was made available for animal feed (goats and sheep). As a result, how much of these pesticides will combine to cause harm to consumers is still unknown. Due to their difficulty in being digested or eliminated, these residues may eventually accumulate in the body of the animal. Pesticide residue levels in goats and sheep can increase to toxic levels as they consume more tainted Khat leaves. These findings suggested that the remaining Khat used as goat and sheep feed included p,p'-DDT and its metabolite p,p'-DDE, which might bioaccumulate and potentially contaminate table foods as a result of these endocrine disruptors. The researcher suggested several areas for further studies on the potential risks associated with consuming table foods derived from goats and sheep fed leftover Khat. First, a quantitative analysis should be conducted to determine the levels of p,p'-DDT and p,p'-DDE in the milk and meat of these animals. This analysis provides a better understanding of the extent of contamination in these animal products. Additionally, investigating the transfer dynamics of these endocrine disruptors from leftover Khat to the milk and meat of goats and sheep would be crucial. This research will help determine whether these contaminants accumulate in animal tissues and at what rate. Furthermore, conducting a human health risk assessment is essential for evaluating the potential health effects of consuming milk and meat from animals fed with leftover Khat containing p,p'-DDT and p,p'-DDE. Assessing exposure levels and determining the potential risks associated with these contaminants are crucial for protecting public health.

Furthermore, if Khat leaves with pesticide residue are not disposed of appropriately, they could damage the soil. Plants, beneficial microorganisms, and other ecosystem occupants could be threatened by pesticide residue that contaminates the soil. This can cause soil fertility to deteriorate and possibly have a detrimental effect on the ecosystem as a whole. Pesticide residues that are broken down or remain inside a plant or animal may be released back into the environment when the plant or animal decays or dies.

4.4 Comparison of results obtained from the leftover Khat samples with MRL values

The average concentration of p,p'-DDT and its metabolite p,p'-DDE found in all leftover Khat samples in the present study (Table 6) was less than the most recent FAO/WHO maximum residue limit (MRL) of 100–200 µg/kg for various agricultural food items (FAO/WHO Food Standards Codex Almintarius, 2014) or an EC MRL value of 50 µg/kg and a Japanese MRL value of 200 µg/kg. However, the remaining Khat samples from Saka, Kersa (Beda Buna), and Mana (Buture) contained p,p'-DDT and p,p'-DDE contamination. It is challenging to determine whether the average concentration of p, p'-DDT and its metabolite p, p'-DDE is insufficient to pose a concern to animals that are used as food for humans, such as goats and sheep despite being below the most current maximum residue limit (MRL).

4.5 Chewable and leftover Khat samples contaminated with pesticide residues

The percentages of chewable and leftover Khat samples with P, P'-DDT and P, P'-DDE pesticide residues were 76.6% and 88.3%, and 69% and 86%, respectively. The highest percentage of Khat samples that were chewable (tender leaves) contained P, P'-DDT and its metabolite P, P'-DDE pesticide residues compared with the percentage of leftover (mature leaves) Khat samples.

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study investigated the level of OCP residue in Khat plants, focusing on both tender (chewable) and mature (leftover) leaves in southwestern Ethiopia. OCP residues on plants might persist despite proper pesticide use in accordance with Good Agricultural Practice. The analysis method was validated before these contaminants were detected to ensure suitability. Parameters such as the LOD, LOQ, % recovery, repeatability, and reproducibility were assessed. The results of the validation indicate that the method is reasonably suitable for the analysis of selected OCPs.

The results of this study indicate that commercially available chewable and leftover Khat samples contained P, P'-DDT and P, P'-DDE pesticide residues. The concentrations of p,p'-DDE and p,p'-DDT in chewable Khat were in the range of 34.07–45.39 and 11.17–34.15 µg/kg, respectively, and the concentrations of p,p'-DDE and p,p'-DDT in leftover Khat were in the range of 30.12–45.04 and 22.21–32.16 µg/kg, respectively. A high concentration of the metabolite (p,p'-DDE) compared to the precursor compound (p,p'-DDT) revealed the historical use of DDT at the study site.

However, the average concentrations of P, P'-DDT and P, P'-DDE in both the chewable and leftover Khat samples in the present study were less than the FAO/WHO maximum residue limits (MRLs) of 100–200 µg/kg for various agricultural food items (FAO/WHO Food Standards Codex Almintarius, 2014) or an EC MRL value of 50 µg/kg and a Japanese MRL value of 200 µg/kg.

The analysis indicated that the chewable Khat samples were contaminated with two organochlorine pesticides, p,p'-DDT and p,p'-DDT; thus, it is clear that the consumption of Khat, which contains unsafe amounts of pesticide residues, is a public health concern and consequently entails additional health costs. By the same token, this may affect economic development. Moreover, the analysis indicated that the leftover Khat samples used as feed for sheep and goats are contaminated with two organochlorine pesticides, P, P'-DDT and P, P'-DDE; thus, unsafe levels of pesticide residues in animal feed pose risks to both the consuming animals and humans who eat products such as meat, milk, and wool from these animals. Thus, the cumulative effect and synergistic toxic effect of these pesticides on consumers should be determined.

5.2 Recommendations

The quality perception of any fresh product by the consumer on the market is based on visual appearance and physical conditions. The attractive fresh and tender leaves of Khat are vibrant green in color and have a soft, velvety texture. They are plump and succulent, with a slight sheen that indicates their freshness. The leaves are delicately fragrant, with a subtle herbal aroma that is both invasive and refreshing. Hence, these products are perceived as high-quality produce and rejected by the consumer. In contrast, damaged and unattractive fresh and tender leaves of Khat are wilted and discolored, and the consumer perceives a dull and lifeless appearance as low quality produce and rejection. Consumers usually believe that fresh Khat leaves are safe, especially when the leaves are grown locally. However, chemical contamination is usually not perceived by observation or even by test. Similarly, in this research, fresh chewable (tender leaves) and leftover (mature leaves) Khat plants, which are attractive, vibrant green in color and have a soft, velvety texture, were found to be contaminated by organochlorine pesticides.

Thus, several issues and questions that need to be addressed were drawn from this research and are described as follows.

- Further investigations are needed to monitor the level of OCPs even in other food samples in the zone.
- Customers and sellers are strongly advised to wash their leaves before consuming them to reduce the risk of pesticide exposure.
- Public awareness should be initiated about the pollution status of both chewable and leftover Khat.
- The MRL of different pesticide residues should be set and enforced.
- It is strongly advised to avoid using Khat leaves contaminated with pesticides as feed for animals or compost for gardens. These contaminated leaves can pose serious health risks to both animals and humans. It is recommended that these patients be properly disposed of in hazardous waste facilities.
- Further studies beginning from the origin of the chewable and leftover Khat to the market level are required to map the pollution status of this third largest export item next to coffee and gold. In addition to being a top export earner, it also plays a considerable role in the

local economy, as it is a source of income for many whole sellers, small-scale traders and businesses that depend on it indirectly.

- Future studies should conduct controlled experiments to determine the extent of pesticide accumulation in animals that consume contaminated Khat leaves and the potential health effects on both animals and humans who consume their products.
- Alternate ways of combating pests, such as IPM, should be considered.

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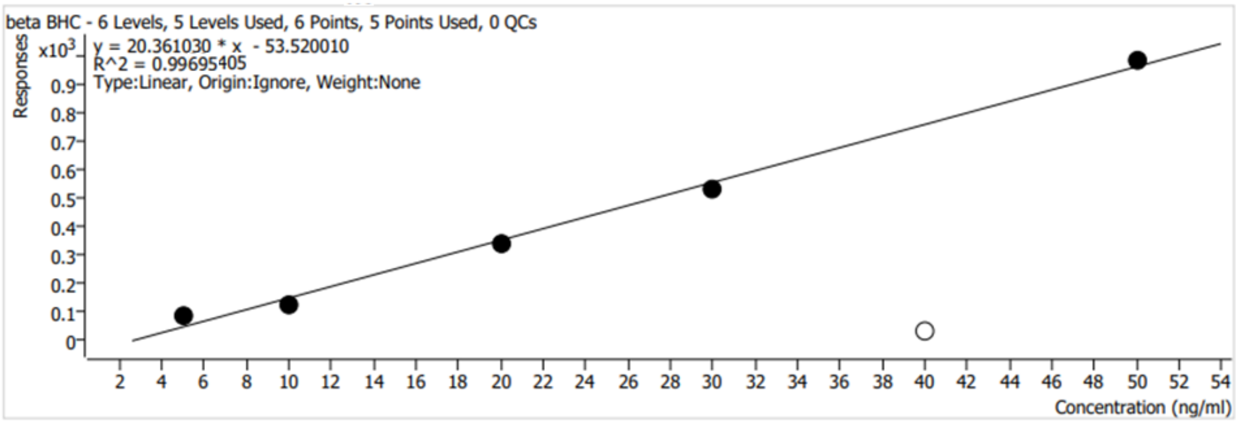
7. APPENDIX

Appendix A: Files during sample collection and extraction time

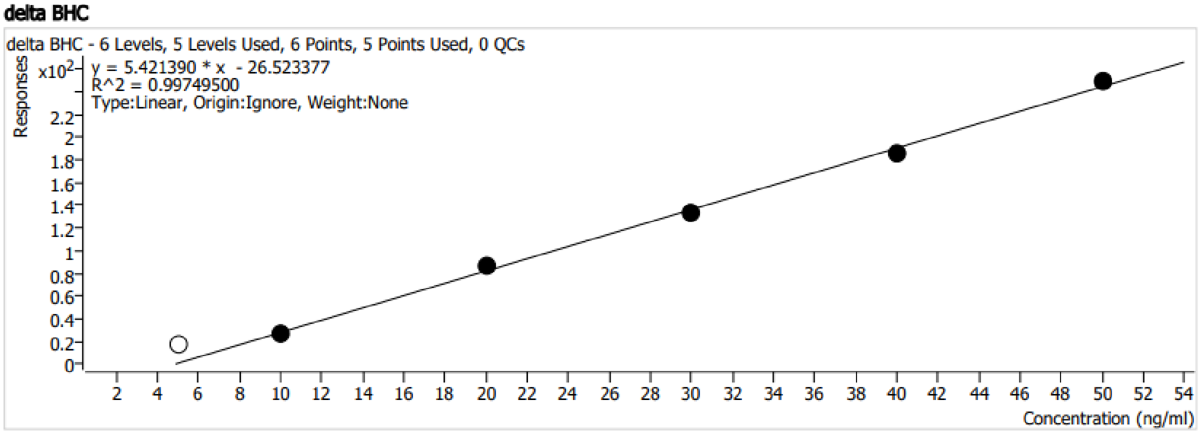
Appendix A. 1: Sample preparation of chewable and leftover Khat samples

Appendix B: Calibration graph with the corresponding equation for each target pesticide

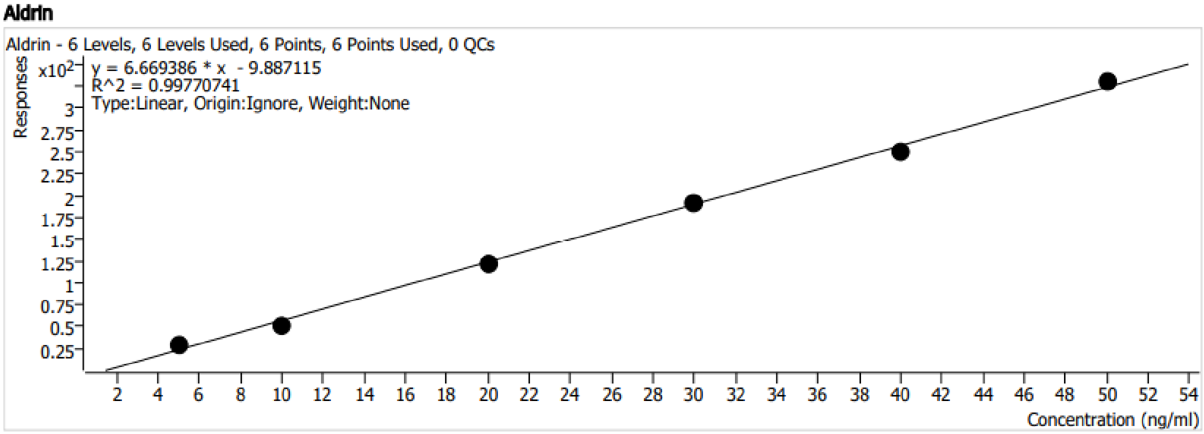
beta BHC



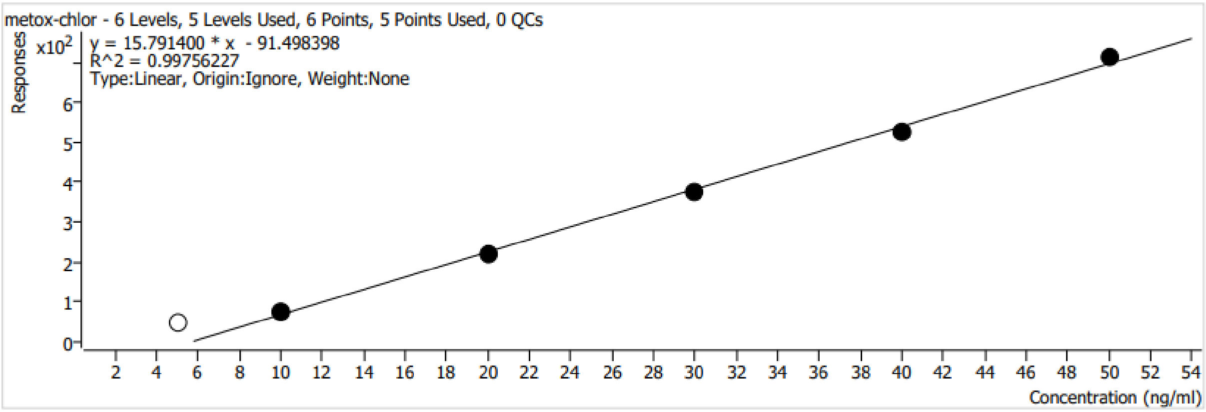
delta BHC



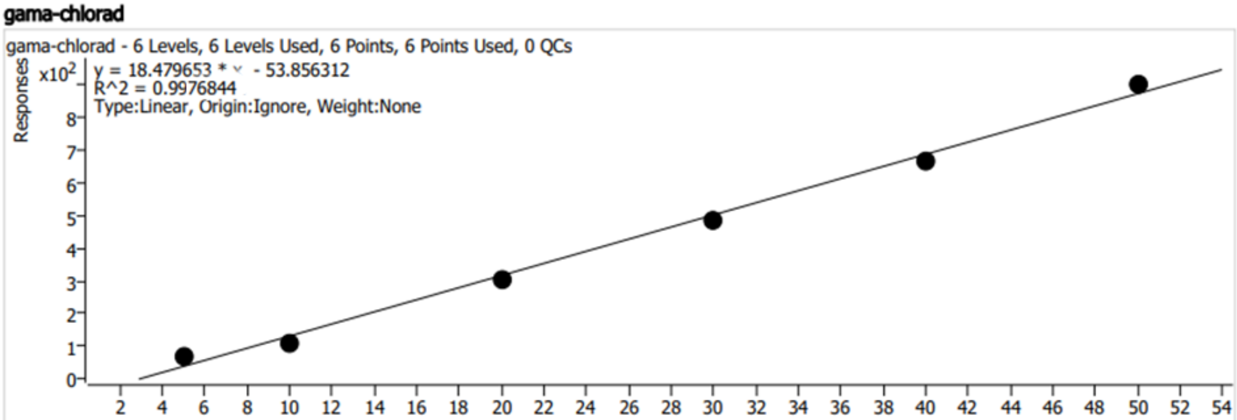
Aldrin



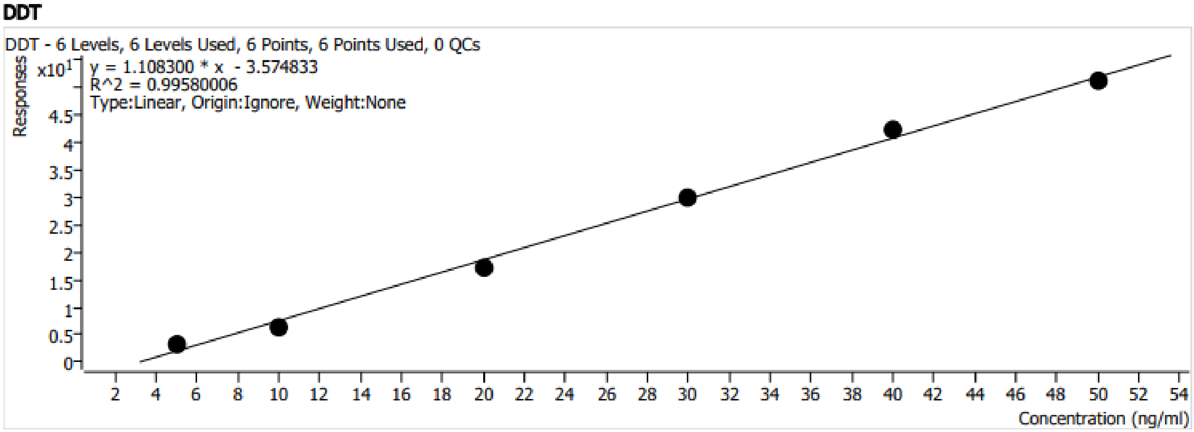
metox-chlor



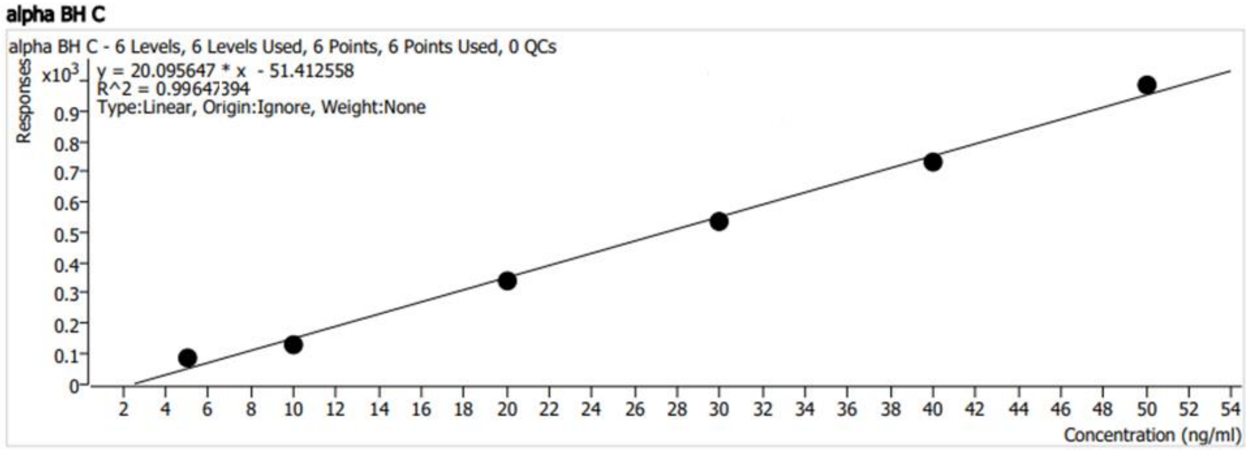
gama-chlorad



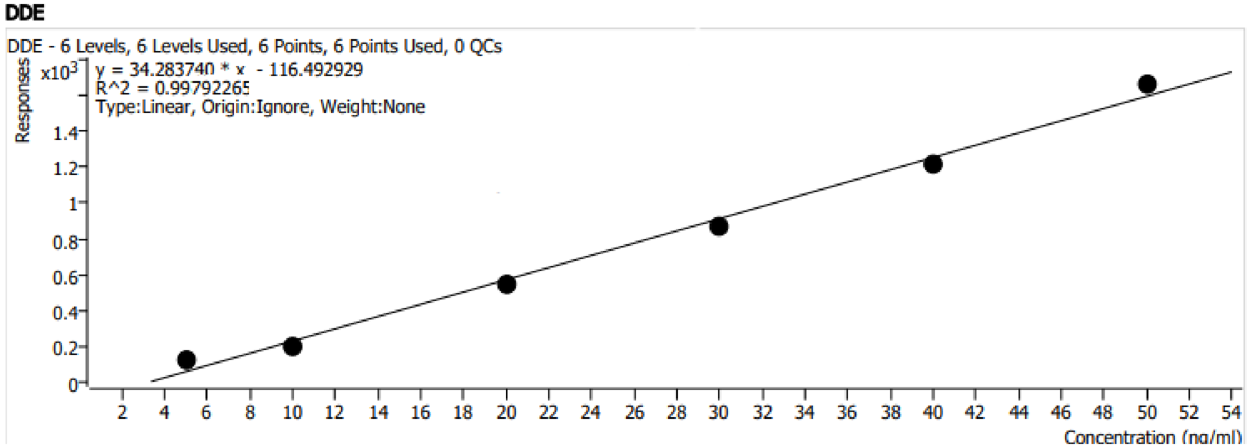
DDT



alpha BH C

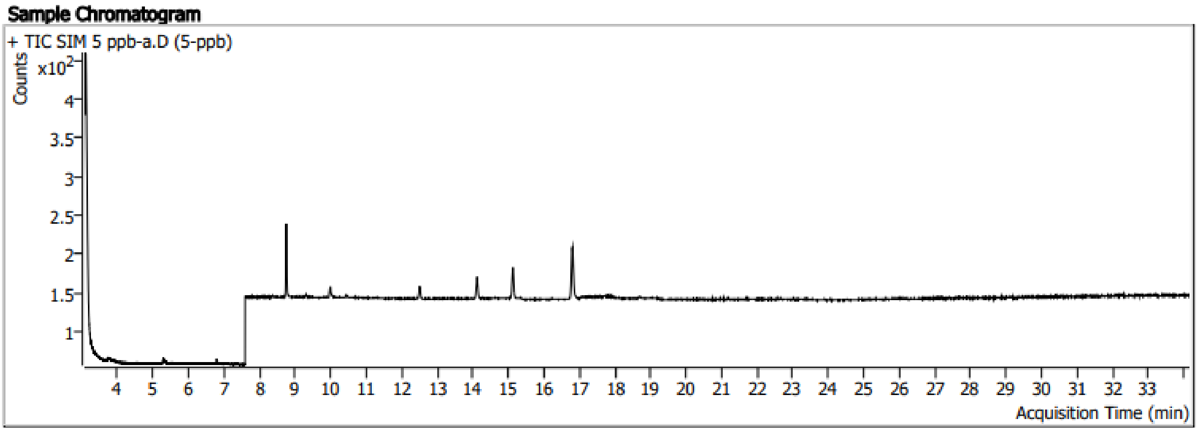


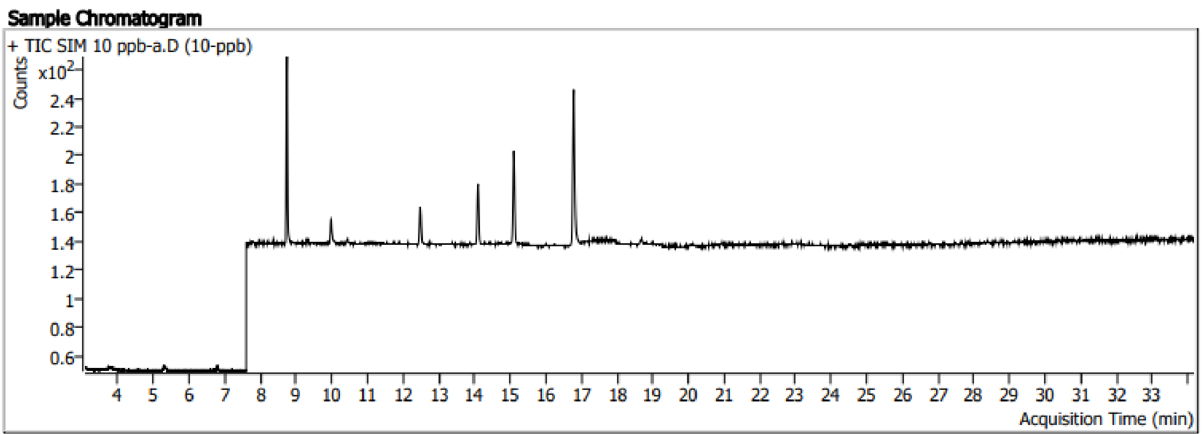
DDE

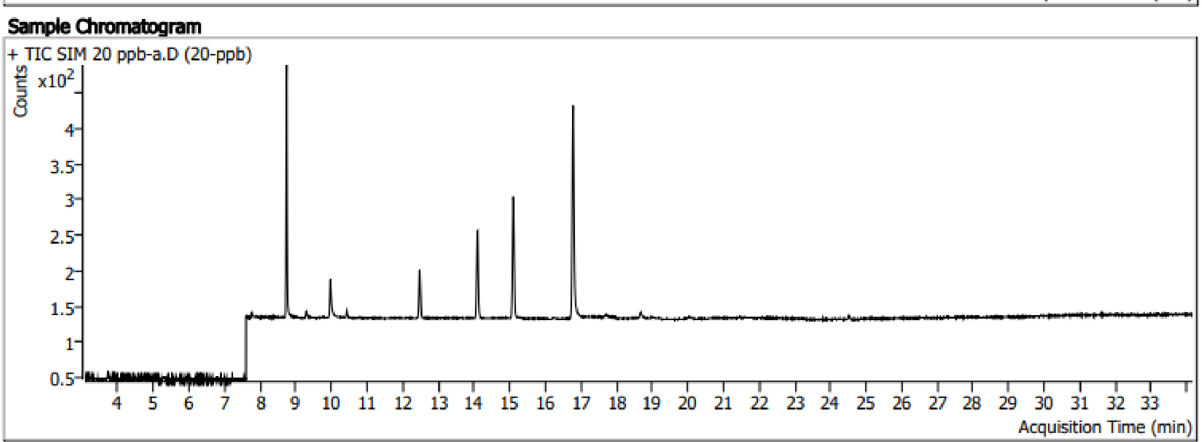


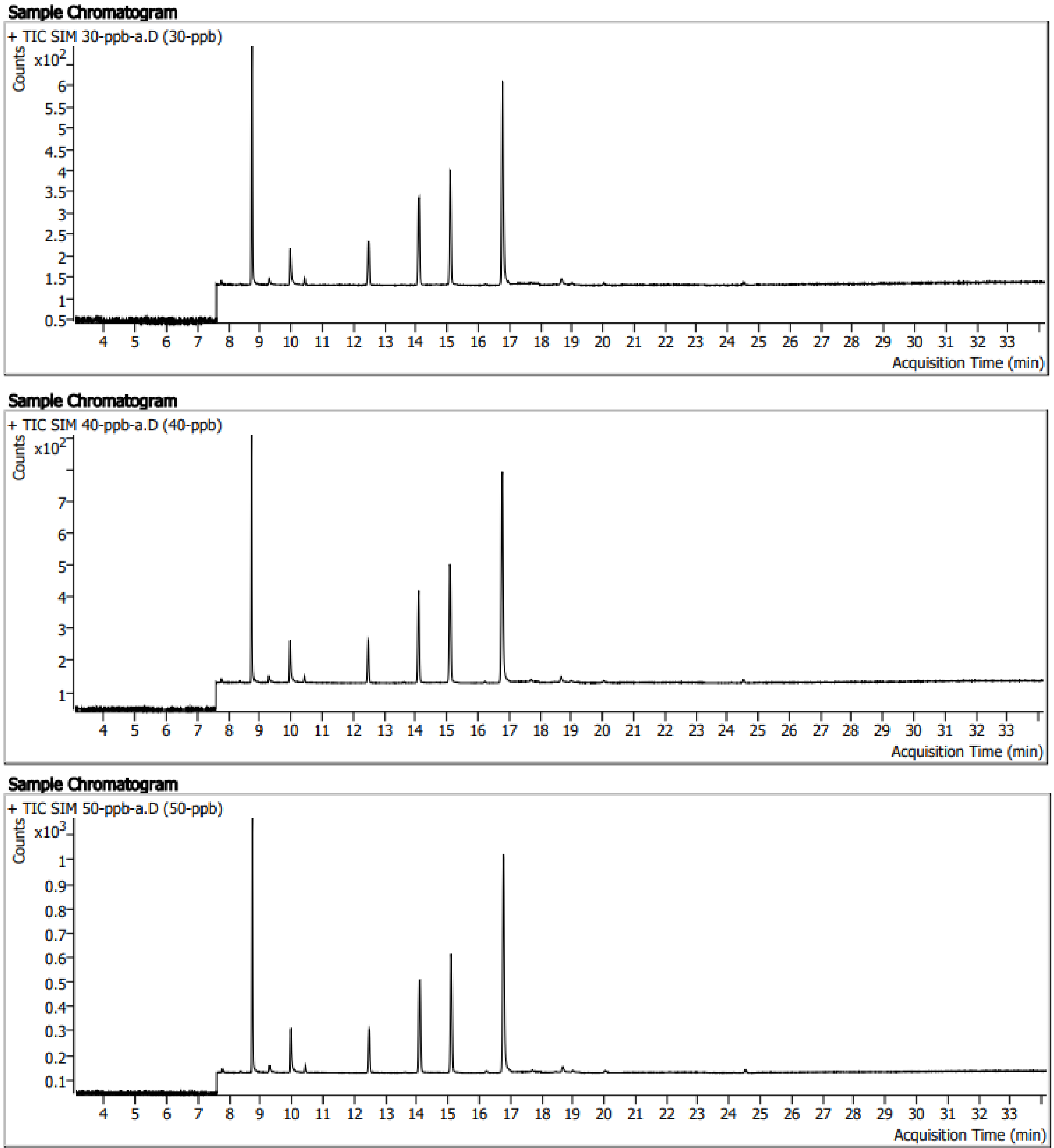
Appendix B. 1: Calibration graphs and equations for each target pesticide analyte

Appendix C: Chromatogram of the studied pesticides









Appendix C. 1: Chromatogram of pesticide standards with pure hexane solvent

Appendix D: Analysis of Pesticide Concentration Variations Among the Four Sites: One-way ANOVA Results in the Appendix

Appendix D. 1: Analysis of variance for p,p-DDT and p,p-DDE between Dedo and Seka samples

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	474.95	0.00
Residual	6.00	0.00	0.00		
Total	7.00	0.00			

	Coefficients	*Standard Error*	*t Stat*	*P value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.00	0.00	0.37	0.73	0.00	0.00	0.00	0.00
Saka	0.92	0.04	21.79	0.00	0.82	1.02	0.82	1.02

Appendix D. 2: Analysis of variance for p,p-DDT and p,p-DDE between Dedo and Beda Buna (Kersa) Khat samples

ANOVA

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	47.03	0.00
Residual	6.00	0.00	0.00		
Total	7.00	0.00			

	Coefficients	*Standard Error*	*t Stat*	*P value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.00	0.00	0.64	0.54	0.00	0.01	0.00	0.01
Beda Buna	1.10	0.16	6.86	0.00	0.71	1.49	0.71	1.49

Appendix D. 3: Analysis of variance for p,p-DDT and p,p-DDE between Dedo and Jiren Khat samples

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	816.08	0.00
Residual	6.00	0.00	0.00		
Total	7.00	0.00			

	Coefficients	*Standard Error*	*t Stat*	*P value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*

Intercept	0.00	0.00	-0.13	0.90	0.00	0.00	0.00	0.00
Jiren	5.50	0.19	28.57	0.00	5.03	5.98	5.03	5.98

Appendix D. 4: Analysis of variance for p,p-DDT and p,p-DDE between Dedo and Buture (Mana) Khat samples

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	6358.45	0.00
Residual	6.00	0.00	0.00		
Total	7.00	0.00			

	Coefficients	*Standard Error*	*t Stat*	*P value*	*Lower 95%*	*Upper 95%*	*Lower 95.0 %*	*Upper 95.0 %*
Intercept	0.00	0.00	-0.20	0.85	0.00	0.00	0.00	0.00
Buture	0.76	0.01	79.74	0.00	0.74	0.79	0.74	0.79

Appendix D. 5: Analysis of variance for p,p-DDT and p,p-DDE between Seka and Bedabuna (Kersa) Khat samples

ANOVA

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	108.35	0.00
Residual	6.00	0.00	0.00		
Total	7.00	0.00			

	Coefficients	*Standard Error*	*t Stat*	*P value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.00	0.00	0.64	0.54	0.00	0.00	0.00	0.00
Beda Buna	1.23	0.12	10.41	0.00	0.94	1.52	0.94	1.52

Appendix D. 6: Analysis of variance for p,p-DDT and p,p-DDE between the Seka and Jiren Khat samples

ANOVA

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	149.90	0.00
Total	7.00	0.00			

	Coefficients	*Standard Error*	*t Stat*	*P value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.00	0.00	-0.13	0.90	0.00	0.00	0.00	0.00
Jiren	5.85	0.48	12.24	0.00	4.68	7.02	4.68	7.02

Appendix D. 7: Analysis of variance for p,p-DDT and p,p-DDE between the Seka and Buture (Mana) Khat samples

ANOVA

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	290.92	0.00
Residual	6.00	0.00	0.00		
Total	7.00	0.00			

	Coefficients	*Standard Error*	*t Stat*	*P value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.00	0.00	-0.20	0.85	0.00	0.00	0.00	0.00
Buture	0.82	0.05	17.06	0.00	0.70	0.93	0.70	0.93

Appendix D. 8: Analysis of variance for p,p-DDT and p,p-DDE between the Beda Buna (Kersa) and Jiren Khat samples

ANOVA

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	28.74	0.00
Residual	6.00	0.00	0.00		
Total	7.00	0.00			

	Coefficients	*Standard Error*	*t Stat*	*P value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	0.00	0.00	-0.13	0.90	-0.01	0.01	-0.01	0.01
Jiren	4.30	0.80	5.36	0.00	2.34	6.26	2.34	6.26

Appendix D. 9: Analysis of variance for p,p-DDT and p,p-DDE between the Beda Buna (Kersa) and Buture (Mana) Khat samples

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1.00	0.00	0.00	1992.01	0.00
Residual	6.00	0.00	0.00		
Total	7.00	0.00			

	Coefficients	*Standard Error*	*t Stat*	*P value*	*Lower 95%*	*Upper 95%*	*Lower 95.0 %*	*Upper 95.0 %*
Intercept	0.00	0.00	0.20	0.85	0.00	0.00	0.00	0.00
Buture	0.14	0.00	44.63	0.00	0.13	0.15	0.13	0.15