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# Organochlorine pesticide residues level in khat and possible human health risks to chewers in Wondogenet district, Sidama regional state, Ethiopia

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To prevent khat plant from destruction of pests and increase its productivity, farmers use different types of pesticides. In Ethiopia, khat is chewed by millions everyday mainly by youngsters. Since it is chewed by many, it is believed that a significant residue level of pesticides to be found in chewable parts of khat which result in adverse effect among the chewing population. The aim of the present study was assessing the organochlorine pesticide residues concentration of on chewable parts of khat and its possible human health risks on chewers. Thirty edible parts of khat samples were collected from both non irrigated and irrigated farms in Wondogenet district. Extraction and cleanup process of pesticide residue of khat samples were undertaken following Quick, Easy, Cheap, Effective, Rugged and Safe extraction (QuEChERS) method. Concentrations of pesticide residue were determined using Gas Chromatography-Mass Spectrometry (GCMS)/Mass Spectrometry (MS) and non-carcinogenic health risk assessments and carcinogenic risk assessments were estimated. Among the 20 targeted Organochlorine Pesticides (OCPs) for analysis in khat samples from non-irrigated farms in this study, 14 residues were detected. The concentrations of the detected OCPs ranged from 0.021 µg/kg to 25.69 µg/kg. Among the analyzed OCPs in khat samples from irrigated farms, 15 residues were detected. Concentration of detected OCPs ranged 0.055 µg/kg to 53.392 µg/kg.

Two pesticides in khat have been found to exceed the default European Commission (EC) maximum limit. These pesticides were endrin aldehyde and methoxychlor. With the mean residue of endrin aldehyde in khat was 25.69 µg kg<sup>-1</sup>, which was twice that of European Union Maximum Residue Limit (EU-MRL) (10 µg kg<sup>-1</sup>). The mean concentration of methoxychlor in khat was 10.608, which was higher than that of EU-MRL (10 µg kg<sup>-1</sup>). The Target Hazard Quotient (THQ) for endrin aldehyde was greater than 1 which shows the non-carcinogenic health risk associated with endrin aldehyde among chewers of khat. The Carcinogenic Hazard Index (CHI) values obtained from chewing khat at irrigated farms were less than 1 but at non-irrigated sites were greater than one (CHI>1) for high consumption probability. This result shows that the chewing of khat from non-irrigated sites at high end consumption probability poses significant non-carcinogenic health hazards. The Potential Carcinogenic Risk (PCR) and Hazard Ratio (HR) values obtained in the present study were less than one for OCPs in both non-irrigated and irrigated farms. This indicates that chewing of khat from both non-irrigated and irrigated farms have no carcinogenic risk to human health. Therefore, it is imperative to monitor pesticide level in khat and policy makers are advised to take an appropriate action at this alarming level to safeguard people from the treat of pesticide pollution from irrigated and non-irrigated areas.

**Key Words:** Khat; QuEChERS extraction method; Organochlorine pesticides; Pollution

## INTRODUCTION

Khat is a plant with green leaves that is a member of the *Celastraceae* family and is endemic to East Africa and Arabian Peninsula. Khat has different names in different countries based on their origin [1]. For example, according to Abebe et al., [2], it is called chat in Ethiopia, miraa in Kenya, qat in Yemen, jaad in Somalia, muhulo in Tanzania and hagigat in Israel. Khat contains a stimulant, the cathinone, that causes better sociability, pleasure, loss of appetite and mild excitement. Usually, the leaves or the soft part of the stem of the plant is chewed with fried peanuts or sugar for its stimulating effect. Contrary to its stimulation aspect of natural content of khat, multiple health problems of khat chewing were reported including effects on cardiovascular system, nervous system, oral cavity, digestive system and genitourinary system [3-7].

During production, khat is attacked by different destructive pests. To prevent destruction of the plant by pests and to preserve their quality and increase productivity, farmers use different pesticides, including Dichloro Diphenyl Trichloroethane (DDT) and other Organo-Chlorine Pesticides (OCPs) [8].

In Ethiopia, khat is chewed by millions of people every day. According to the Ethiopian Demographic and Health Survey (EDHS), the life time of khat chewing was 26.9 % among males and 12.1% among female Ethiopian adults. Another study reported the prevalence of khat chewing was 23.61% in Ethiopia [9]. According to the same survey, two men and three women chewed khat for six or more days in a month. In addition, a study was

conducted in the city of Nekemte East Wollega region and showed that khat chewing prevalence is currently 48.6 % [10].

Farmers in many parts of Ethiopia said they often used DDT and others, either by themselves or mixed with the rest of the poisonous pesticides to be rid of khat pests. In a khat expansion study in Wondogenet, Southcentral Ethiopia, it was found that the khat farmers used different types of toxic pesticides to control the insects and sprayed them about 2 times a week on Khat [11].

The characteristics of Organochlorine Pesticides (OCPs) are high toxicity, delayed breakdown and bio-accumulation, hence, causes health effects including, endocrine disorders, neurological damage and the ecology is impacted when environmental contamination with organochlorine pesticides occurs [12]. Because of the harm they bring to humans and the environment, the stockholm convention forbids the use of nine OCPs for agricultural purposes namely DDT, dieldrin, mirex, heptachlor, endrin, hexachlorobenzene, chlordane and aldrin, toxaphene [13]. Even though, these OCPs are banned for use, significant residue levels of the pesticides are reported in different environmental compartments that could be due to their slow degradation nature and illegal use. It has been documented that organochlorine chemicals found in water, soil, honey and vegetables [14-16].

In Ethiopia, there are few studies on the level of OCPs residue on khat, of which studies that were discovered that some of these studies reported p,p'-DDT concentrations ranged from 0.010 to 0.026 mg/kg, 10.8 to 19.71 g/kg,

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0.0323 mg/L to 0.0001 mg/L, 4.77 mg/kg to 5.77 mg/kg, 141.2 to 999.01 g/kg in khat samples in different areas of the country [17-21]. However, as far as the researcher knowledge there is a few studies on organochlorine pesticide residues on chewable parts of khat. Furthermore, the previous studies on OCPs residue on khat focused mainly on DDT and its metabolites. Therefore, the main aim of this study was to determine the concentrations of 20 organochlorine pesticide residues on chewable parts of khat and assess possible human health risk to chewers in Wondogenet district, Sidama National Regional State, Ethiopia.

## MATERIALS AND METHODS

### The study area

The study was conducted in Wondogenet district, located in Sidama National Regional State, Ethiopia and it is located approximately 28.9 km from Hawassa city and 265 km from Addis Ababa, the capital city of Ethiopia. Based on Ethiopia's population projections, Wondogenet district had a total population of 2,40,182 in 2023. It is located at 7°06'-7°07'N latitude and 38°37'-38°42'E longitude, with an altitudinal range between 1600 and 2580 m above sea level [22]. The climate of the study area is characterized by the Woyna Dega agro-climatic type. The average annual rainfall and temperature of the study area are 1210 mm and 20°C, respectively. Wondogenet is one of Ethiopia's most potential khat-producing areas, particularly in the Sidama region. All kebeles in the district produce khat from which some use irrigation to cultivate it, while others don't. Farmers in these study areas use pesticides to control pests on the khat plant and increase its productivity.

### Sampling and sample collection

Wondogenet district is purposefully selected for our study among high khat producing districts in the Sidama region, Ethiopia. A total of two kebeles in the Wondogenet district among 15 kebeles, known for high khat production, were purposely selected to collect khat samples. One of the kebeles used irrigation for producing khat, while the second did not (non-irrigated farms). In irrigated farms, farmers harvest khat multiple times using irrigation water compared to those who don't use it. Thirty composite khat samples were gathered from irrigated and non-irrigated farms between July and September of 2023. Ten farmers field (5 from each non irrigated and irrigated farms), were selected purposely based on the size of their larger size of their khat producing area. Ten different khat fields were sampled in triplicate, with five composite samples from each farm. From each field, seven subsamples were collected in triplicate. To create a representative sample, a 0.5 kg sample was produced by collecting samples from five individual plants in every corner of a particular field using zigzag method with 1 m apart and homogenized to represent the bulk sample.

All the collected samples, one from each field, were packed, labeled and placed in a polyethylene bags and stored in the refrigerator at 4°C for one day before transporting to Addis Ababa. The samples were then transported in an insulated icebox, to Jije Laboglass Pvt. Ltd. Company in Addis Ababa for examination of Organochlorine (OC) pesticides contamination and then stored in the dark at 4°C until further analysis.

### Sample preparation

The chewable portions of the khat samples that were collected were dried under shade and milled with a mortar and pestle. The ground samples were stored in labeled polyethylene bags and refrigerated at 4°C till additional examination.

### Sample extraction

Extraction and cleanup process of pesticide residue was undertaken following the Quick, Easy, Cheap, Effective, Rugged and Safe extraction method (QuEChERS) [23]. A 50 mL centrifuge tube was filled with 5 g of homogenized khat sample. After agitating the tubes for 30 seconds, a 10 ml aliquot of water was supplied using a dispenser and the tubes were then allowed to hydrate for thirty minutes. Each tube was then filled with a 10 mL volume of acetonitrile, sealed and violently shaken for a minute. Each tube received a direct addition of 1.5 g sodium acetate and 6 g magnesium sulfate anhydrous from the Bond Elut QuEChERS Association of Official Agricultural Chemists (AOAC) extraction salt. To make sure the solvent was thoroughly mixed with whole *Catha edulis* sample and that the crystal aggregates were suitably split apart, after tightly sealing the tubes, they were given a minute of vigorous shaking. At last, sample tubes underwent a 4,500

rpm centrifugation.

### Dispersive solid phase extraction cleanup

A 15-milliliter bond elut QuEChERS AOAC tube was filled with 2 milliliters of the acetonitrile layer. The dispersive Solid Phase Extraction (dSPE) tube included 400 mg Primary Secondary Amine (PSA), 1200 mg of anhydrous magnesium sulfate, 400 mg C18 and 400 mg Graphitized Carbon Black (GCB). Additionally, each tube received two milliliters of n-hexane. After firmly capping the tubes, they were vortexed for a minute. After that, the tubes were spun for five minutes at 4,500 rpm using a normal centrifuge. Ultimately, a 2 ml auto-sampler vial was filled with an aliquot of the top hexane layer in preparation for GC-MS injection.

### Pesticides residues analysis method (GCMS/MS analysis)

A total of 20 organochlorines pesticides residues were targeted to determine their concentration in khat and, we used a GC Agilent 7890B equipped with an auto-sampler G4513A connected to an Agilent Technologies, Inc., CA, USA, triple quadrupole mass detection system operating at 7000C GC/MS. To enable analyte dissociation and very passive movement channel into the sensor, a 30-meter long capillary GC column with an internal diameter of 0.25 mm and a film thickness of 0.25 µm was utilized, manufactured by Agilent J and W DB-5ms ultra inert.

Helium (99.999%) was used as the carrier gas, with 1.2 mL/min for the column flow rate. At 280°C, the injector and interface were set up for splitless injection of 1 µL. After passing over a transfer line at 280°C, the GC column's eluent was supplied to a 70 eV source of ionization by electron impact, which had 280°C temperature of the source. A selected ion monitoring mode was employed in the examination. Instrumental responses were analysed conducted using Agilent Technologie's Mass Hunter software.

### Method validation

The organochlorine pesticide residues found in the khat samples were estimated making use of a calibration curve created using reference combination of 20 OCPs 5, 20, 40, 60 and 200 ng/g. The validation result fulfills with the SANTE 113122021 v2 Guidelines, as shown in Table 1 [24]. Limit of Quantifications (LOQs) and Limit of Detection (LODs) were determined for the method's validation. The values of LOD and LOQ were computed as 3.3 and 10 times intercept's standard deviation over slope of the regression line's respectively [25]. The recoveries of every analyte in every sample were calculated using the concentration attained with the substance's initial concentration [26]. Every analyte's calibration curves had a linear distribution between 5 and 200 ng/g of concentration according to method validation. For every pesticide, the determination regression coefficient the range of ( $R^2$ ) values was 0.9887 to 0.9999. The LOD and LOQ of all detected OCPs ranged from 0.004 to 0.050 and 0.011 to 0.152, respectively. The percentage recoveries of all OCPs were in the acceptable range (98.8 and 105.89%) except for methoxychlor olefin and with a Relative Standard Deviation (RSD) of less than 20%, all pesticide residues exhibit satisfactory accuracy (Table 1).

**TABLE 1**

**Method validation parameter results in khat residue analysis**

Pesticides	R <sup>2</sup>	Recovery (%)	LOD	LOQ	%RSD
DDE	0.9998	99.3	0.006	0.018	7.76
DDT	0.9999	98.81	0.004	0.011	3.57
DDD	0.999	103.9	0.01	0.032	8.24
Aldrin	0.9952	102.4	0.028	0.085	8.27
Diendrin	0.9966	99.78	0.023	0.071	7.01
endosulfanI	0.997	102.4	0.022	0.067	1.83
EndosulfanII	0.9965	101.8	0.024	0.072	2.25
Endosulfan sulfate	0.9974	102	0.02	0.062	6.29
Endrin	0.9957	99.43	0.027	0.081	7.94
Endrin ketone	0.9988	101.78	0.014	0.042	4.55

Endrin aldehyde	0.9847	112.37	0.05	0.152	1.34
Alpha BHC	0.9999	100.72	0.004	0.011	0.68
Beta BHC	0.9994	103.57	0.01	0.03	5.76
Delta BHC	0.9997	99.45	0.006	0.021	4.06
Gamma BHC	0.9947	99.85	0.029	0.089	2.04
Heptachlor epoxy isomer B	0.9981	99.33	0.018	0.054	5.29
Heptachlor	0.9941	103.47	0.031	0.094	10.74
Methoxychlor olefin	0.9887	164.63	0.043	0.131	0.402
chlordane cis	0.9967	105.89	0.023	0.07	8.04
Chlordane trans	0.9969	101.15	0.023	0.068	7.84

**Note:** LOD: Limit of Detection in µg/kg; LOQ: Limit of Quantification in µg/kg; RSD: Residual Standard Deviation; Recovery%: Mean recovery; DDE: Dichloro Diphenyldichloro Ethylene; DDT: Dichloro Diphenyl Trichloroethane; DDD: Dichloro Diphenyl Dichloroethane; BHC: Benzene Hexachloride; R<sup>2</sup>: Regression coefficient.

#### Data quality assurance

Sampling of representative khat samples from each sampling site, using certified analytical techniques as well as applying standard processes with graded products was used for the purpose of maintaining quality data.

#### Methods of possible human health risk assessment for chewers

The Maximum Residue Limits (MRLs) of pesticides for leafy vegetables, as suggested by Codex Alimentarius and European Commission, were compared to the data for pesticide residue levels. The Health Risk Index (HRI), the Cancer Benchmark Concentration (CBC) and the Estimation Daily Intake (EDI) were employed to compute both carcinogenic and non-carcinogenic human health risks assessments for each OC pesticide leftovers throughout the edible sections of khat. To calculate the long-term risks to chewers' health, the Estimated Daily Intakes (EDIs) of pesticide residue and food consumption assumptions were employed. The EDI was calculated by multiplying the mean residual pesticide concentration (mg/kg) in the food of interest by the food consumption rate (kg/d) and then dividing the resultant product by the body weight of the consumers [27]. The calculation was based on the assumption that a 60 kg adult would ingest 0.1 kg of average daily consumption rate [18].

$$EDI = \frac{c_x c_r}{kg \text{ body weight}} \dots\dots\dots (1)$$

Where, c is the mean concentration of pesticide residue (mg kg<sup>-1</sup>), BW is body weight (kg), EDI is for approximate daily consumption (mg kg<sup>-1</sup> d<sup>-1</sup>) and c<sub>r</sub> is intake rate.

#### Non carcinogenic human health risk assessment

The health risk index was determined by dividing the estimated daily intake by the corresponding values of acceptable daily intake and assuming that an adult weighs 60 kg was used to determine the non-carcinogenic health risk [28]. According to Akoto et al., [29], a food item is deemed hazardous to consumers if its health risk index is greater than 1 and acceptable if it is less than 1. Codex Alimentarius provided the Acceptable Daily Intake (ADI) values for all OC pesticides.

$$HI = \frac{EDI}{ADI} \dots\dots\dots (2)$$

To estimate the Cumulative Hazard Index (CHI), the following equation (3) was used as described in Refstrup et al., [30].

$$CHI = \frac{EDA_1}{ADI_1} + \frac{EDA_2}{ADI_2} + \frac{EDI_n}{ADI_n} = \sum \frac{EDI_i}{ADI_i} \dots\dots\dots (3)$$

Where, EDI<sub>1</sub>, EDI<sub>2</sub>, EDI<sub>n</sub> and EDI<sub>i</sub> are the approximate daily dosages of each insecticide. For any pesticide, the permissible daily intake or reference is ADI<sub>1</sub>, ADI<sub>2</sub>, ADI<sub>n</sub> and ADI<sub>i</sub>.

Using the below formula (4), the Hazards Ratio (HR) was calculated to ascertain the effects of carcinogens [31].

$$HR = \frac{EDI}{CBC} \dots\dots\dots (4)$$

Cancer benchmark concentration was obtained following the equation below by Javier Perera-Rios et al., [32].

CBC is calculated as follows:

$$\frac{Risk \times weight \text{ of body (kg)}}{Chewing \text{ rate of khat } (\frac{kg}{d}) \times OSFs} \dots\dots\dots (5)$$

Carcinogenic effects, Cancer Benchmark Concentration (CBC) was obtained by setting the risk to one in one million owing to lifetime exposure, where risk is the highest permissible risk level (1 × 10<sup>-6</sup>). United states environmental protection agency provided the Oral Slope Factor (OSF) of insecticides.

#### Potential carcinogenic risk assessment

To assess health risk posed through chewing of khat the cancer risk estimates and Hazard Ratios (HRs) were used.

The cancer risk of the studied OCP was also evaluated using the formula [33].

$$PCR = C \times CSF \dots\dots\dots (6)$$

Where, PCR is Probable Cancer Risk, C is mean concentration of pesticide, CSF is Cancer Slope Factor.

## RESULTS AND DISCUSSION

#### Concentrations of OCPs residues in khat

Among the 20 targeted OCPs for analysis in khat samples from non-irrigated farms in this study, 14 residues were detected and the six undetected were alpha BHC and endrin ketone endrin, chlordane trans, heptachlor and methoxychlorolefin. Concentrations of the detected OCPs ranged from 0.021 µg/kg to 25.69 µg/kg. Among the detected the highest concentration 25.69 µg/kg was for andrin aldehyde and the lowest 0.021 µg/kg was for delta BHC (Table 2). Whereas, khat samples from irrigated farms, 15 residues were detected and five undetected were delta BHC, alpha BHC, endrin ketone, endrin aldehyde and chlordane cis. Concentration of detected OCPs ranged 0.055 µg/kg to 53.392 µg/kg. Among the detected the highest concentration 53.392 µg/kg was for methoxychlor olefin and the lowest 0.0055 µg/kg (Table 2).

Two pesticides in khat have been found to exceed the default EC maximum limit, as shown in Table 2. For serious pollution and food safety concerns, only foods with pesticide residues in excess of the European Union Maximum Residue Limit (EU MRL) default were considered. The mean residue of endrin aldehyde in khat was 25.69 µg kg<sup>-1</sup>, which was twice that of EU-MRL (10 µg kg<sup>-1</sup>). Two pesticides in khat have been found to exceed the default EC maximum limit for leaf vegetables, as shown in Table 2. The mean concentration of methoxychlor in khat was 10.608, which was higher than that of EU-MRL (10 µg kg<sup>-1</sup>).

Among all the OCPs detected in khat samples in both irrigated and non-irrigated agricultural areas, the highest concentration recorded was endrin aldehyde. But the concentration of Parent compound endrin was very low and its metabolite endrin ketone was not detected (Table 2). The difference in mean concentration of endrin aldehyde between non irrigated (25.603 µg kg<sup>-1</sup>) and irrigated farms may be due to the past application of pesticides by farmers in the non-irrigated farm site and farmers from irrigated farm did not applied endrin. Moreover, the present study revealed that 100% of the samples in non-irrigated farm site contained eldrin aldehyde. In comparison with European commission maximum residue limit the mean concentrations of total endrin in khat samples from non-irrigated farm locations was higher than the most recent Maximum Residue Levels (MRLs) for spinach, a leafy vegetable, set by the European commission (0.01 mg/kg). As a result, chewing khat from our study area may be unsafe due to eldrin aldehyde contamination.

TABLE 2  
The concentration (µg/kg) of OCPs residues in chewable parts of khat

Detected OCPs	Concentrations (µg/kg ) of OCPs in khat and frequency of occurrence (%)					
	Samples from non-irrigated farms			Samples from irrigated farms		
	Range	Mean ± standard deviation	Frequency	Range	Mean ± standard deviation	Frequency
Delta BHC	0.069-0.111	0.021 ± 0.037 <sup>a</sup>	25	-	ND <sup>b</sup>	-
Gamma BHC	1.585-3.057	1.495 ± 1.562 <sup>a</sup>	50	2.092-4.508	1.208 ± 1.560 <sup>a</sup>	38.9
Beta BHC	3.192-5.208	1.008 ± 1.825 <sup>a</sup>	25	3.901-4.359	0.229 ± 0.973 <sup>a</sup>	5.6
Alpha BHC	-	ND <sup>a</sup>	-	-	ND <sup>a</sup>	-
Total BHC	4.846-8.376	2.54 ± 3.424	-	5.993-8.867	1.437-2.533	-
Endrin ketone	-	ND <sup>a</sup>	-	-	ND <sup>a</sup>	-
Endrin aldehyde	23.643-27.743	25.693 ± 0.659 <sup>a</sup>	100	-	ND <sup>b</sup>	-
Edrin	-	ND <sup>a</sup>	-	1.108-2.892	0.892 ± 0.922 <sup>b</sup>	50
Chloridene cis	4.009-10.591	3.291 ± 3.453 <sup>a</sup>	50	-	ND <sup>b</sup>	-
Chlordane trans	-	ND <sup>a</sup>	-	2.326-3.674	0.674 ± 1.072 <sup>b</sup>	50
Heptachlor	-	ND <sup>a</sup>	-	2.764-5.396	1.316 ± 1.916 <sup>b</sup>	33.3
Heptachlor_epoxide_isomer B	1.357-2.243	0.443 ± 0.81 <sup>a</sup>	25	1.98-2.619	0.639 ± 0.930 <sup>a</sup>	33.3
Dieldrin	0.570-0.930	0.180 ± 0.326 <sup>a</sup>	25	0.844-1.556	0.356 ± 0.521 <sup>a</sup>	33.3
Aldrin	1.062-4.525	0.328 ± 0.595 <sup>a</sup>	25	1.341-1.859	0.259 ± 0.596 <sup>a</sup>	16.7
Endosulfan_I	6.442-10.558	2.058 ± 3.724 <sup>a</sup>	25	6.912-9.608	1.348 ± 3.102 <sup>a</sup>	16.7
Endosulfan_II	3.628-10.032	3.202 ± 3.352 <sup>a</sup>	50	4.051-7.969	1.959 ± 2.852 <sup>a</sup>	33.3
Endosulfan_sulphate	1.232-2.428	0.598 ± 0.883 <sup>a</sup>	33.3	0.295-0.405	0.055 ± 0.127 <sup>a</sup>	16.7
Total endosulfan	11.302-23.018	5.858 ± 7.959	-	11.258-17.982	3.3125 ± 6.081	-
p,p'-DDE	1.67-4.29	1.620 ± 0.985 <sup>a</sup>	83.3	1.058-6.342	2.642 ± 1.245 <sup>b</sup>	83.3
p,p'-DDD	12.73-25.33	19.030 ± 2.156 <sup>a</sup>	100	17.658-27.458	22.558 ± 1.774 <sup>b</sup>	100
p,p'-DDT	4.311-16.431	10.371 ± 2.235 <sup>a</sup>	100	5.294-27.306	11.006 ± 5.323 <sup>a</sup>	100
Total DDT	18.711-46.051	31.021 ± 5.376	-	23.958-61.106	36.206 ± 8.342	-
Methoxychlor_olefin	-	ND <sup>a</sup>	-	53.392-74.608	10.608 ± 24.409 <sup>a</sup>	16.7

**Note:** Values with different letters with in a row are significantly different at p<0.005 level range value shows minimum and maximum data values; DDE: Dichloro Diphenyldichloro Ethylene; DDT: Dichloro Diphenyl Trichloroethane; DDD: Dichloro Diphenyl Dichloroethane.

Khat samples in both non irrigated and irrigated farm sites showed the mean concentration sequence of DDT: p,p'-Dichloro Diphenyl Dichloroethane (DDD)>p,p'-DDT>p,p'-Dichloro Diphenyldichloro Ethylene (DDE). The present study revealed that 100% of khat samples contained p,p'-DDT. Similarly, recent study in South Wolo, Ethiopia revealed that 100% of the khat samples contained p,p'-DDT, on the contrary, other studies in south western and eastern Ethiopia revealed lower frequencies of p,p'-DDT occurrences of 66.7% and 61% respectively [17,21,32]. The highest mean concentration of p,p'-DDD was reported next to eldrin aldehyde, where concentration of p,p'-DDT and p,p'-DDE were relatively low. This may be due to degradation of parent compound p,p'-DDT to p,p'-DDD. The mean and range of total DDT concentrations in khat samples collected from non-irrigated and irrigated farm sites were 31.021 (18.71-46.05) and 36.21 (23.96-61.11), respectively. This value indicates the difference in level of use of pesticides by farmers. The detected residual concentrations of metabolites of DDT, p,p'-DDD and p,p'-DDE were significantly different among the non-irrigated and irrigated sites khat samples.

The present study found that the mean concentrations of total DDT for khat samples in both irrigated and non-irrigated farm locations (Table 1) were lower than the latest Maximum Residue Levels (MRLs) for leafy vegetables (0.05 mg/kg) and agricultural foods (0.1 mg/kg) set by the European

Commission (EC) and Food and Agriculture Organization (FAO)/ World Health Organization, (WHO) respectively. Compared to the findings with similar previous studies conducted throughout Ethiopia, the concentrations of total DDT in khat in present study were comparable to those data recorded from Sidama (16.7-44.81 g kg<sup>-1</sup>) by Ligani et al., [18]. However, it was lower than those reported in South Wollo (9.42-247 µg kg<sup>-1</sup>) by Woldetsadik et al., [34], Jimma (41.4-149 µg kg<sup>-1</sup>) by Mekonen et al., [17], Gelemso (755 µg kg<sup>-1</sup>) by Daba et al., [21] and Haramaya (111 µg kg<sup>-1</sup>) by Regassa et al., [35].

To determine whether the contamination was the result of recent application or past usage, the ratios between the parent analyte and their metabolites have often been utilized. In that regard, the ratios for DDT was (p,p'-DDD+p,p'-DDE)/p,p'-DDT [36,37]. According to Calamari et al., [36] ratio less than one may indicate current application of DDT, whereas a value larger than one was indicative of past application of DDT. The finding of the present study revealed that the ratio (p,p'-DDD+p,p'-DDE)/p,p'-DDT was greater than one for the khat samples from both non-irrigated and irrigated farm areas. This indicates that there was past application of DDT and it has been used for long periods of time in our study area. In another way, the results also indicated that the ratio of DDT, (p,p'-DDD+p,p'-DDE)/p,p'-DDT for present study sites was 2.0 for non-irrigated farm whereas it was 2.287 for irrigated farm site. Comparatively, lower ratios ranging from 0.2-1.06 and



0.55-1.27 for DDT in khat were recorded in South Wollo and Sidama, respectively. These findings suggest that DDT might have been extensively used in khat production in Sidama area including the present study area and in South Wollo. However, higher ratios were recorded in the Jimma zone (4.1-7.0) indicating a recent using practice of DDT in khat production in this area.

Three isomers of Benzene Hexachloride (BHC) were also detected in khat in different concentration among different farms (Table 2). Their concentration in khat ranged from 0.069-0.111  $\mu\text{g kg}^{-1}$  for delta BHC, 1.585-4.508  $\mu\text{g kg}^{-1}$  for gamma BHC and 3.192-5.208  $\mu\text{g kg}^{-1}$  for beta BHC were occurred in 25%, 50% and 25% of khat samples respectively. Among the isomers of BHC, beta BHC is higher in khat samples, whereas the frequency of occurrence of gamma BHC was highest in samples. The findings of this study was lower than the compared with the study on khat in East Hararge which detected only the isomer alpha BHC (10.37  $\mu\text{g kg}^{-1}$ ) [19]. The cause for this difference may be due to different application of pesticides in the two study areas. In present study, the concentration of total BHC was much lower than that of total DDT; this disparity could be the difference in the preferences of pesticides by farmers.

Two isomers of endosulfan (alpha and beta endosulfan) and its degradation product (endosulfan sulphate) were detected in khat samples from both irrigated and non-irrigated farm sites in the present study area. They are found in order endosulfan II (3.202  $\mu\text{g kg}^{-1}$ ) > endosulfan I (1.959  $\mu\text{g kg}^{-1}$ ) > endosulfan sulfate (0.598  $\mu\text{g kg}^{-1}$ ) in non-irrigated farm and endosulfan II (3.202  $\mu\text{g kg}^{-1}$ ) > endosulfan I (1.348  $\mu\text{g kg}^{-1}$ ) > endosulfan sulfate (0.5  $\mu\text{g kg}^{-1}$ ) in irrigated farm site. The concentration of total endosulfan in khat samples in the present study was lower than the EC tolerable limits of 0.05 mg for leafy vegetables, this finding was inconsistent to other finding of the study on khat samples in Sub-Humid region of Ethiopia [38].

Aldrin was also found in small quantity in khat samples in both irrigated 0.259  $\mu\text{g kg}^{-1}$  and non-irrigated farm sites (0.328  $\mu\text{g kg}^{-1}$ ) which were ranged from 1.341  $\mu\text{g kg}^{-1}$ -1.859  $\mu\text{g kg}^{-1}$  and 1.062  $\mu\text{g kg}^{-1}$ -4.525  $\mu\text{g kg}^{-1}$ , respectively. The finding of the present study was comparable with the previous study done by Adamu et al., [19] who reported 2.53  $\mu\text{g kg}^{-1}$  concentration of aldrin in khat samples. However, the study done by Daba et al., [21] has reported the level of aldrin was below detection limit. The residue level of aldrin in the current study was below MRLs for leafy vegetables set by EC and FAO/WHO 0.01 mg/kg and 0.05 mg/kg, respectively.

The present study showed that the mean concentration of dieldrin in khat samples from non-irrigated and irrigated farm sites were 0.180  $\mu\text{g kg}^{-1}$  and 0.356  $\mu\text{g kg}^{-1}$ , respectively which were lower than MRLs set for leafy vegetables by EC (0.01 mg/kg) and FAO/WHO 0.05 mg/kg. Dieldrin was not detected in east Hararge khat samples according to report by Adamu et al., [19].

The mean concentration of heptachlor in khat samples of irrigated farm was 1.316  $\mu\text{g kg}^{-1}$  but it was not detected in non-irrigated farm sites and the concentration for its isomer B in khat samples from non-irrigated and irrigated samples were 0.598  $\mu\text{g kg}^{-1}$  and 0.639  $\mu\text{g kg}^{-1}$  respectively. The concentration of total heptachlor in the present study was much lower than MRLs values set by EC which is 0.01 mg/kg for leaf vegetables. The current investigation found a lower concentration of Heptachlor in khat samples than the mean value reported 6.7  $\mu\text{g kg}^{-1}$ , in east Hararge by Adamu et al., [19]. In contrast, the mean content of heptachlor in our study was greater than that of an investigation on cabbage in India which reported below quantification limit [39].

The mean concentration of chlordane cis in khat samples in non-irrigated farm was 3.291  $\mu\text{g kg}^{-1}$  and chlordane trans was 0.674  $\mu\text{g kg}^{-1}$  in khat samples of irrigated farm sites. The mean concentration of total chlordane in khat samples in the present study was lower than MRLs limit set by EC 0.01 mg/kg for leafy vegetables. In the present study methoxychlor olefin was not detected in khat samples in non-irrigated farm site but in irrigated farm the mean concentration was 10.608  $\mu\text{g kg}^{-1}$ . The present finding indicates that the mean concentration of methoxychlor olefin in irrigated farm site was higher than MRLs set by European commission, 0.01 mg/kg for leafy vegetable.

#### Possible human health risk assessment methods

The non-carcinogenic risk assessments are given below:

**Hazard quotient:** The organochlorine pesticides detected in very small concentration were excluded in estimation of exposure and risk assessment.

Accordingly, in this study we examined the risk assessment for nine OCPs. The highest intakes of total DDT (258.5 ng/kg/day) was obtained from high end consumption probability for non-irrigated farm khat samples (Table 3). These estimated daily intake value was far lower than a value obtained in south western Ethiopia, Jimma (2000 ng/kg/day). However, comparable estimated daily intake values were reported from chewing of khat collected from different locations across Sidama Ethiopia (24.2-325 ng/Kg/day) and for adults from Kemisse in South Wollo, Ethiopia (401 ng/kg/day). Thus, consumption rate and data format were variable among these studies, this comparison with the finding by Mekonen et al., [17] was qualitative, since they investigated exposure to DDT from khat consumption by taking 19.2 g/kg/day. On the other hand EDI of total DDT and p,p'-DDD from chewing of both irrigated and non-irrigated khat higher than those.

Non-carcinous risk estimates revealed that Target Hazard Quotient (THQ) values for p,p'-DDT, p,p'-DDD, total DDT, endosulfan I, endosulfan II, total endosulfan chlordane cis and methoxychlor olefin were less than 1, indicating that chewing khat from both non irrigated and irrigated farms may not pose non-carcinogenic health risks to chewers of khat. This finding does not guarantee safety of chewers because farmers were using these pesticides for other food items including cabbage. Whereas, HQ value was greater than one for endrin aldehyde for khat samples from non-irrigated farm site indicating that endrin aldehyde contributed the most to non-cancer risk among all OCPs in case of adults on high consumption probability. Besides this, the health quotient values for chewers of khat from non-irrigated farms for p,p'-DDT, p,p'-DDD, total DDT, endrin aldehyde, endosulfan I, endosulfan II, total endosulfan, chlordane cis and methoxychlorolefin ranged from 0.0006 to 0.214 for low end consumption probability and 0.0045 to 1.071 for high end consumption probability. This result finds THQ value greater one for endrin aldehyde showing that chewing khat from non-irrigated farms may pose non carcinogenic health risks at high end (500 g) consumption probability.

The HQ value for irrigated farm for p,p'-DDT, p,p'-DDD, total DDT, endrin aldehyde, endosulfan I, endosulfan II, total endosulfan chlordane cis and methoxychlor\_olefin ranged from 0.0001768 to 0.00603 for low end consumption probability and 0.00089 to 0.03017 for high end consumption probability. HQ values less than one (HQ<1) were reported in both cases, indicating that chewing khat from current research sites can not pose non-carcinogenic health risks for both low end consumption probability and high end consumption probability.

**Cumulative risk assessment:** The Cumulative Health Index (CHI) of a mixture of pesticides is the sum of the Hazard Quotient (HQ) values, which are calculated as the ratio of the estimated daily pesticide intake to the acceptable daily intake [29]. The CHI values obtained from chewing khat at irrigated farm sites and low consumption probability at non irrigated sites were less than one (CHI<1). The findings indicate that the consumption of all pesticide residues evaluated in this study through chewing khat at the both low end and high end consumption probabilities from each site cannot cause potentially insignificant non carcinogenic health hazards. The CHI values obtained from chewing khat at non irrigated sites were greater than one (CHI>1) for high consumption probability. This result shows that the chewing of khat from non-irrigated sites at high end consumption probability poses significant non carcinogenic health hazards.

**Potential carcinogenic risks:** The cancer risk assessment (Table 4) shows that p,p'-DDT, p,p'-DDD and chlordane cis have Possible Cancer Risk (PCR) values that are less than 1 (<1). This result indicates that chewing of khat from our study areas have no carcinogenic risk to human health. The cancer risk assessment shows the PCR value for total DDT, endrin aldehyde, endosulfan I, endosulfan II, total endosulfan and methoxychlor\_olefin was not considered because oral slope factor for these pesticides was not available under united states environmental agency integrated risk assessment list.

**Hazard ratios:** In our study hazard ratios for cancer risk assessments for concentrations of p,p'-DDT, p,p'-DDD and chlordane cis for both samples from irrigated and non-irrigated sites were less than one (Table 5). The hazard ratios values for OCPs follows a sequence p,p'-DDT in irrigated farm site > p,p'-DDT in non-irrigated farm site > chlordane cis in non-irrigated farm site > p,p'-DDD irrigated farm site > p,p'-DDD no irrigated farm site (Table 5). The finding of hazard ratios shows that there was no possible cancer risk to human health.

TABLE 3

Estimated daily intakes ( $\mu\text{g/kg/day}$ ) of organochlorine pesticides and Hazard Index (HI) of potential non carcinogenic effects from the consumption of 100 g (low end) and 500 g (high end) fresh khat leaves

OCP		Values of human health risk assessment indexes							
		Non irrigated farm				Irrigated farm			
		EDI	ADI	THQ	HR	EDI	ADI	THQ	HR
p,p'-DDT	100 g	$1.7 \times 10^{-2}$	10	$1.7 \times 10^{-3}$	N	0.183	10	$1.8 \times 10^{-3}$	N
	500 g	$8.64 \times 10^{-2}$	10	$8.64 \times 10^{-3}$	N	$9.17 \times 10^{-2}$	10	$9.17 \times 10^{-3}$	N
p,p'-DDD	100 g	$3.17 \times 10^{-2}$	10	$3.17 \times 10^{-3}$	N	$3.75 \times 10^{-2}$	10	$3.75 \times 10^{-3}$	N
	500 g	0.16	10	$1.585 \times 10^{-2}$	N	0.188	10	$1.879 \times 10^{-2}$	N
Total DDT	100 g	$5.17 \times 10^{-2}$	10	$5.17 \times 10^{-3}$	N	$6.03 \times 10^{-2}$	10	$6.03 \times 10^{-3}$	N
	500 g	0.26	10	$2.585 \times 10^{-2}$	N	0.302	10	$3.017 \times 10^{-2}$	N
Endrin aldehyde	100 g	$4.28 \times 10^{-2}$	0.2	0.214	N	-	-	-	-
	500 g	0.21	0.2	1.071	Y	-	-	-	-
Endosulfan I	100 g	$3.43 \times 10^{-3}$	6	$5.7 \times 10^{-4}$	N	$2.24 \times 10^{-3}$	6	$3.74 \times 10^{-4}$	N
	500 g	$8.575 \times 10^{-2}$	6	$1.429 \times 10^{-2}$	N	$1.123 \times 10^{-2}$	6	$1.87 \times 10^{-3}$	N
Endosulfan II	100 g	$5.3 \times 10^{-3}$	6	$8.89 \times 10^{-4}$	N	$3.265 \times 10^{-3}$	6	$5.442 \times 10^{-4}$	N
	500 g	$2.6 \times 10^{-2}$	6	$4.447 \times 10^{-3}$	N	$1.6325 \times 10^{-2}$	6	$2.72 \times 10^{-3}$	N
Total endosulfan	100 g	$9.76 \times 10^{-3}$	6	$1.627 \times 10^{-3}$	N	$5.52 \times 10^{-3}$	6	$9.21 \times 10^{-4}$	N
	500 g	$4.8816 \times 10^{-2}$	6	$8.136 \times 10^{-3}$	N	0.278	6	$4.600 \times 10^{-2}$	N
Chlordane cis	100 g	$5.485 \times 10^{-3}$	0.5	0.11	N	-	-	-	-
	500 g	$2.7425 \times 10^{-2}$	0.5	$5.485 \times 10^{-2}$	N	-	-	-	-
Methoxycyclohex- olefin	100 g	-	-	-	-	$1.768 \times 10^{-2}$	100	$1.768 \times 10^{-4}$	N
	500 g	-	-	-	-	$8.9 \times 10^{-2}$	100	$8.9 \times 10^{-4}$	N
Hazard index	100 g	-	-	0.238	N	-	-	$1.3596 \times 10^{-2}$	N
	500 g	-	-	1.203	Y	-	-	$6.821 \times 10^{-2}$	N

**Note:** OCP: Organo-Chlorine Pesticides; EDI: Estimated Daily Intake; ADI: Acceptable Daily Intake; THQ: Target Hazard Quotient; HR: Health Risk; DDE: Dichloro Diphenyldichloro Ethylene; DDT: Dichloro Diphenyl Trichloroethane; DDD: Dichloro Diphenyl Dichloroethane.

TABLE 4

Cancer risk assessment of organochlorine pesticides in khat samples from non-irrigated and irrigated farms

		Mean	Oral slope factor	PCR	CR
p,p'-DDT	Non irrigated	$1.0371 \times 10^{-2}$	0.34	$3.52614 \times 10^{-3}$	No
	Irrigated site	$1.1006 \times 10^{-2}$	0.34	$3.74204 \times 10^{-3}$	No
p,p'-DDD	Non irrigated	$1.903 \times 10^{-2}$	0.24	$4.5672 \times 10^{-3}$	No
	Irrigated site	$2.2558 \times 10^{-2}$	0.24	$5.41392 \times 10^{-3}$	No
Total DDT	Non irrigated	$3.1021 \times 10^{-2}$	-	-	NC
	Irrigated site	$3.62 \times 10^{-2}$	-	-	NC
Endrin aldehyde	Non irrigated	$2.5693 \times 10^{-2}$	-	-	NC
	Irrigated site	-	-	-	NC
Endosulfan I	Non irrigated	$2.058 \times 10^{-3}$	-	-	NC
	Irrigated site	$1.348 \times 10^{-3}$	-	-	NC
Endosulfan II	Non irrigated	$3.202 \times 10^{-3}$	-	-	NC
	Irrigated site	$1.959 \times 10^{-3}$	-	-	NC

# Organochlorine pesticide residues level in khat and possible human health risks to chewers in Wondogenet district, Sidama regional state, Ethiopia

Total endosulfan	Non irrigated	$5.858 \times 10^{-3}$	-	-	NC
	Irrigated site	$3.3125 \times 10^{-3}$	-	-	NC
Chlordane cis	Non irrigated	$3.91 \times 10^{-3}$	0.35	$1.3685 \times 10^{-3}$	NO
	Irrigated site	ND	0.35	-	NC
Methoxycychlor_olefin	Non irrigated	ND	-	-	NC
	Irrigated site	$1.0608 \times 10^{-2}$	-	-	NC

**Note:** NC: Not Calculated : No: No cancer risk OSF values were obtained from USEPAIRIS (2023); DDE: Dichloro Diphenyldichloro Ethylene; DDT: Dichloro Diphenyl Trichloroethane; DDD: Dichloro Diphenyl Dichloroethane.

**TABLE 5**  
Hazard ratios values for carcinogenic risk assessments for p,p'-DDT, p,p'-DDD and chlordane\_cis

	OCP	EDI (mg/kg)	CBC	HR
p,p'-DDT	Non irrigated	$1.7 \times 10^{-6}$	$1.76 \times 10^{-3}$	$9.63 \times 10^{-4}$
	Irrigated site	$3.75 \times 10^{-6}$	$1.76 \times 10^{-3}$	$1.07 \times 10^{-2}$
p,p'-DDD	Non irrigated	$3.17 \times 10^{-6}$	$2.5 \times 10^{-2}$	$1.48 \times 10^{-4}$
	Irrigated site	$3.17 \times 10^{-6}$	$2.5 \times 10^{-2}$	$1.5 \times 10^{-4}$
Chlordane cis	Non irrigated	$5.485 \times 10^{-7}$	$1.71 \times 10^{-3}$	$3.20 \times 10^{-4}$
	Irrigated site	-	-	-

**Note:** OCP: Organochlorine Pesticides; EDI: Estimated Daily Intake; CBC: Cancer Bench mark Concentration; HR: Hazard Ratios; DDT: Dichloro Diphenyl Trichloroethane; DDD: Dichloro Diphenyl Dichloroethane.

## CONCLUSION

The current study investigated residue levels and potential health risks of 20 organochloride pesticides in khat samples from non-irrigated and irrigated farms of wondogenet district of Ethiopia. Among the analyzed samples, 18 pesticides were detected but 2 of them were not detected at all. Among the detected pesticides endrin aldehyde in khat non irrigated farm site and methoxychlor olefin in khat from irrigated farm sites were exceeded the maximum residue limit set by European commission. Overall the study suggests that there are possible risks to human health from organochlorine pesticide contamination in these khat plants. The level of OCP pollution found in khat is the cause for the concern while non-carcinogenic risk values for khat samples from all the farms were <1 and therefore the health risk assessment value did not indicate any immediate danger to human health, except for endrin aldehyde in non-irrigated farms that it can pose health risk from endrin aldehyde contamination to chewers in high end consumption probability because its calculated HQ value shows >1. The commutative risk assessment also shows (CHI)>1 for khat samples in non-irrigated farms while it shows <1 for irrigated farms, therefore the study suggests that there are possible non carcinogenic health risk for chewers of khat from organochlorine contamination in non-irrigated farms but not for irrigated farms. In contrast, the calculated cancer risk estimates and HRs of the khat samples indicated that the chewing of khat does not cause cancer as PCR and HR for cancer risk of p,p'-DDD, p,p'-DDT and chlordane cis was less than one. Therefore, it is imperative to monitor pesticide level in khat and policy makers are advised to take an appropriate action at this alarming level to safeguard people from the treat of pesticide pollution from irrigated and non-irrigated areas.

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