

Multi Residue Analysis of Pesticides in Wheat and Khat Collected from Different Regions of Ethiopia

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Abstract The present study investigated the presence and level of pesticide residues in wheat and khat samples collected from various localities of Ethiopia. The khat samples from Galemso and Aseno had *p,p'*-DDT concentrations ranged from 141.2 to 973.0 µg/Kg and 194.3–999.0 µg/Kg, respectively. Diazinon was detected in all the khat samples from BadaBuna (173.9–686.9 µg/Kg) but not in any of the samples from Galemso and Aseno. Diazinon was detected in all the wheat samples obtained from both Arsi and Bale (125.8 and 125.6 µg/Kg, respectively) and aldrin levels in these samples were below the quantification limit. Khat may be a contributing factor in the pathological diseases found among khat users.

Keywords Pesticides · Wheat · Khat · Ethiopia

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The use of pesticides is considered to be indispensable practice for the production of adequate food supply for the increasing world population and for the control of insect-borne diseases (Rissato et al. 2007). Thus contamination of the environment with pesticides and the pesticides entry into the food chain is unavoidable especially in developing countries. Organochlorines that enter the food chain bioaccumulate due to their lipophilicity and remain in ecosystems for a long period of time (Dehn et al. 2005). Monitoring studies from Asia revealed widespread contamination of foodstuff and animal feed with pesticide residues (Seok et al. 2008).

Agricultural intensification, including increased use of pesticides, is considered a viable option in Ethiopia to overcome the problems of increasing population, shrinking arable land and food insecurity. Government extension services promote the use of pesticides to improve the productivity of smallholder agriculture and alleviate food insecurity (Environment and Social Assessment International 2006). Inventories carried out in Ethiopia showed the presence of over 400 stores with 1,500 tones of obsolete pesticides and 1,000 tonnes of contaminated equipment. Poor storage conditions (e.g. leaking drums, burst open sacks) and stock managements lent to a great risk of contamination and posed a great threat to human health and the environment. This led to the situation where Ethiopia was considered to be one of the worst contaminated countries in Africa (Environment and Social Assessment International 2006).

Despite the ban of DDT in the 1970 s and the commitment of the international community to eliminate the use of 12 persistent organic pollutants, including DDT, which was demonstrated by the signage of over 90 countries on the “Stockholm Convention on Persistent Organic Pollutants” in 2001, the use of DDT still continuing in

many part of the world for preventing the spread of malaria due to its effective and relatively inexpensive form of mosquito control. In September 2006, the World Health Organization announced that it would support the use of DDT for malaria control, and as a result some countries including Ethiopia are reconsidering its use (Eskenazi et al. 2008). In spite of their ban for both agricultural and malaria control use in Ethiopia, aldrin and dielderin have been recently found in soil (Westbom et al. 2008). Other organophosphate pesticides, such as diazinon and malathion, are commonly used in Agro-industries and can frequently enter the food chain.

Khat (*Catha edulis* Forsk, Celastraceae) is a perennial shrub that originated in Ethiopia and cultivated in East Africa, Madagascar and the Middle East. Young khat leaves are traditionally chewed to attain a mild euphorical state of mind and stimulant effect among its users (Krizevski et al. 2007) owing to presence of the natural compound Benzoylethanamine (Cathinone) in the plant. Around 10 million people commonly use khat in East Africa and countries in the Arabian Peninsula, but several other European and Polynesian countries allow the importation of khat (Al-Motarreb et al. 2002; Griffiths et al. 2010). As part of their agricultural practices, Khat producers in Ethiopia use pesticides to control insects affecting the plant in addition to its common use for the control of malaria. New patterns of khat consumption, including morning chewing sessions and khat parties, have emerged in the East African countries (Warfa et al. 2007) which may lead to more frequent exposure to pesticides. The consumption of Khat may represent a high risk source of pesticides as the plants are normally chewed fresh without any treatments that can potentially reduce the pesticides concentration (e.g. soaking in boiling water and other thermal treatment).

Wheat is the major staple food in Ethiopia and Ethiopia is the major wheat producer in sub-Saharan Africa. Arsi and Bale regions are the major wheat producing zones of Ethiopia. Despite the documented frequent and occasionally heavy use of pesticides in Ethiopia, no information on the level of pesticide residues in khat and wheat is available. The objective of this study was to investigate presence of pesticide residues in khat and wheat samples from some localities in Ethiopia to explore the level of contaminations and determine the risk potential.

Materials and Methods

Wheat and khat samples analyzed for pesticide residue were collected from different localities in Ethiopia where pesticides are commonly used for agriculture and control of malaria. Wheat samples were collected in December 2008

from the state farms from Lole (East Arsi) (7°57'N 39°7'E) and Sinana (Bale zone) (6.97N39.97E) where pesticides are mainly used for agriculture and Adami Tulu (East Shoa zone) (7°9'N38°7'E) where pesticides are used both for agriculture and malaria control. The khat samples were collected from five different farms each in January 2009 from BadaBuna (Jimma) (7°40'N 36°50'E), Aseno (Butajera) (8°07'N 38°22'E) and East Gelemso (Hararge) (8°49'N 40°31'E), where pesticides are used to control pests and malaria. The three regions are known for high khat production and khat consumption.

Standard pesticides with the corresponding purity: *p,p'* DDT and *o,p'* DDT (99.6%), *p,p'* DDE (99%), Aldrin (98.1%), Diazinon (96.5%) and Malathion (96%) were purchased from Sigma–Aldrich, (Germany). All the solvents and reagents used were of analytical grade or higher.

Pesticide extraction and clean up from khat samples was performed following the procedure described by Huang et al. (2007) with slight modifications. The collected leaf samples were dried under shade and powdered. A 1 g of the powdered sample was mixed with 5.0 mL water and 1.0 g sodium chloride. The mixture was sonicated for 3 min and kept at room temperature for 30 min. Then the mixture was extracted three times with 4.0 mL each of the extraction solvent [acetone : ethyl acetate : n-hexane (1:2:1 v/v/v)] for 3 min followed by centrifugation at 2,500 rpm for 2 min, and the organic phase from the extractions was combined and dried with 1.0 g of anhydrous sodium sulfate. The solution was then filtered and reduced to about 5 mL at 45°C. A 5 mL of the concentrated residual extract was introduced into the Solid Phase Extraction (SPE) column, which was preconditioned with 6.0 mL of the extraction solvent. The retained pesticides on the column were eluted with 6.0 mL of acetone–ethyl acetate mixture (1:2 v/v). The eluent was collected and evaporated to dryness *in vacuo*. Finally, the residue was re-dissolved in 0.5 mL ethyl acetate and used for GC–MS analysis.

The pesticide extraction and clean up procedures from wheat samples were adopted from Walorczyk (2007) with some modifications. Wheat samples were powdered using hand mill and 5 g of the finely powdered material was mixed with 10 mL of water followed by 15 mL acetonitrile. The mixture was sonicated for 5 min and 1 g trisodium citrate dihydrate, 4 g anhydrous sodium sulfate and 1 g sodium chloride were added and the mixture was immediately hand-shaken for 1 min. The resultant mixture was then centrifuged at 2,500 rpm for 5 min. The supernatant liquid was transferred to a polypropylene centrifuge tube containing 0.75 g anhydrous sodium sulfate. The tube was hand shaken for 1 min and centrifuged at 2,500 rpm for 5 min. The supernatant was cleaned up using SPE. The SPE eluent was transferred into a glass test tube containing a 50 µL of 5% formic acid in acetonitrile (v/v). The extract was

Table 1 Mean percent recovery \pm RSD of six pesticides in wheat and khat samples at fortification levels indicated ($n = 3$)

Pesticides	Spiking level (ng/mL)	Wheat		Khat	
		Mean recovery	%recovery \pm RSD	Mean recovery	%recovery \pm RSD
Diazinon	400	387.4	96.9 \pm 2.2	385.9	96.5 \pm 2.3
Malathion	400	374.7	93.7 \pm 3.8	371.0	92.7 \pm 3.1
Aldrin	200	192.3	96.2 \pm 5.5	179.3	89.7 \pm 4.9
<i>p,p'</i> -DDE	200	181.8	90.9 \pm 3.7	175.3	87.6 \pm 1.8
<i>o,p'</i> -DDT	133.7	127.8	95.6 \pm 5.1	127.1	95.1 \pm 8.8
<i>p,p'</i> -DDT	400	381.3	95.3 \pm 2.5	385.7	96.4 \pm 3.6

evaporated to dryness and the residue was re-dissolved in 1.5 mL toluene prior to its injection into the GC–MS system. Gas chromatographic (GC) analysis was carried out using a GC–MS QP 2010 instrument. The mass spectrometer was interfaced to a computer running the software GC MS solution release 2.30 (Shimadizu Corporation Kyoto, Japan). Chromatographic separation was carried out using a DB-5MS fused-silica capillary column (30 m \times 0.25 mm ID, 0.25 μ L film thickness of 95% dimethyl-5% diphenyl poly sil phenylene) from J and W scientific. The gas chromatographic (GC) conditions were set as described by Huang et al. (2007). Helium gas was used as the carrier gas at a flow rate of 1 mL/min. A volume of 1 μ L mixture of standard or sample extract was injected in a splitless mode. The injection port temperature was 260°C and the oven temperature was initially held at 60°C for 2 min and then ramped to 150°C at a rate of 15°C/min, and finally at 6°C/min to 280°C (held for 11 min). The mass spectrometer was operated with an EI source in the selected ion monitoring (SIM) mode. The electron energy was 70 eV and the ion source and the interface temperature was maintained at 230°C. The electron multiplier voltage was 1 kV and solvent delay was set to 15 min.

Spiking recoveries were determined by adding the pesticides to wheat and khat samples at fortification level indicated and recovery assays were done in triplicate (table 1). Quantitative analysis was carried out using triphenyl phosphate (TPP) as internal standard at a concentration of 0.4 μ g/mL. The detection and quantification limits for the studied pesticides were respectively as indicated in the bracket, diazinon (13.9, 46.4 μ g/Kg), malathion (16.7, 55.6 μ g/Kg), aldrin (14.4, 48.0 μ g/Kg), *p,p'*-DDE (13.6, 45.3 μ g/Kg), *o,p'*-DDT (13.7, 45.6 μ g/Kg) and *p,p'*-DDT (14.8, 47.6 μ g/Kg). The BQL is the pesticides levels below the quantification levels stated above.

Results and Discussion

A summary of the pesticide residues found in Khat samples from three different localities of Ethiopia is shown in

Table 2. Malathion was not present in the khat samples and all the studied pesticides were detected at various levels (Table 2). Diazinon was detected in all the khat samples from BadaBuna at high residue levels while diazinon levels in samples from Gelemso and Aseno were below the quantification level. The average diazinon residue level in samples from BadaBuna was 462.6 μ g/kg (range 173.9–751.4 μ g/kg). Diazinon in Ethiopia is registered for the control of pests that attack vegetables and fruits and the high concentrations of residue observed in khat samples from BadaBuna indicate its excessive use in that region. Given that the daily consumption of khat is estimated to be in the range of 100–300 g (Al-Habori 2005), khat chewers in BadaBuna are exposed to acute toxicity of diazinon since the maximum residue level (MRL) under EU-regulation set for vegetables and fruits is 10 μ g/kg. Aldrin was detected below the quantification limit in one of the khat samples from Gelemso. Currently the use of aldrin in Ethiopia is restricted. Therefore, its presence could be from historical application and could be attributed to the persistence of organochlorine pesticides in the environment.

DDT and its metabolites were detected in all khat samples from Aseno and Gelemso. The average total DDT (*o,p'*-DDT, *p,p'*-DDT and *p,p'*-DDE) in Gelemso and Aseno were 755.5 \pm 394.0 and 709.0 \pm 457.9 μ g/kg, respectively. The total DDT in some samples from these regions was much higher than the EU set MRLs for total DDT in edible foodstuffs such as cereals (50 μ g/kg), citrus fruits, vegetables and sugar plants (10 μ g/kg). The highest amount of total DDT observed in two of the Aseno khat samples (1.224 and 1.163 mg/Kg) and the highest total DDT residue detected in one of the khat sample from Gelemso (1.372 mg/Kg) were about 240–1,200 times the EU MRL for foodstuff. Giving the lipophilic nature of organochlorine compounds, foodstuffs having less water and greater lipid contents have greater potential to accumulate the organochlorine pesticides. Because khat has high water and low lipid contents, the obtained results indicate that very high levels of DDT have been used. The high level of DDT in the khat samples instead of DDE suggest that DDT is currently in use for control of pests in

Table 2 Observed pesticides residue level in khat samples collected from Gelemso, Bada Buna and Aseno

Sample	Pesticides concentration (µg/Kg)						
	Diazinon	Malathion	Aldrin	<i>p,p'</i> -DDE	<i>o,p'</i> -DDT	<i>p,p'</i> -DDT	Total DDT
<i>Gelemso</i>							
Sample 1	BQL	ND	BQL	BQL	177.7	592.3	783.9
Sample 2	BQL	ND	ND	ND	230.7	442.8	673.5
Sample 3	ND	ND	ND	ND	399.0	973.0	1,372
Sample 4	BQL	ND	ND	ND	139.3	141.2	280.5
Sample 5	BQL	ND	ND	ND	219.3	448.2	667.5
<i>BadaBuna</i>							
Sample 1	686.9	ND	ND	ND	BQL	ND	BQL
Sample 2	186.2	ND	ND	ND	ND	ND	ND
Sample 3	751.4	ND	ND	ND	BQL	ND	BQL
Sample 4	514.4	ND	ND	ND	ND	ND	ND
Sample 5	173.9	ND	ND	ND	ND	ND	ND
<i>Aseno</i>							
Sample 1	ND	ND	ND	ND	224.8	999.0	1,223.8
Sample 2	ND	ND	ND	ND	68.0	261.9	329.9
Sample 3	ND	ND	ND	ND	117.5	453.9	571.4
Sample 4	ND	ND	ND	ND	62.2	194.3	256.5
Sample 5	BQL	ND	ND	ND	197.5	965.8	1,163.3

BQL Below quantification limit, for diazinon (46.4 µg/Kg), malathion (55.6 µg/Kg), aldrin (48.0 µg/Kg), *p,p'*DDE (45.3 µg/Kg), *o,p'*DDT (45.6 µg/Kg) and *p,p'*DDT (47.6 µg/Kg), *ND* Not detected

the study regions. If the accumulation was the result of historical use, the level of DDE would have been much higher than DDT as DDT gets metabolized to DDE over time. The half life of *p, p'* DDT in soil is 2,000 days (Tadeo 2008). The range for total DDT residue level in khat samples from Gelemso and Aseno were 280.5–1,372 µg/Kg and 256.5–1,163.3 µg/Kg, respectively. This wide range of total DDT residue level indicates the difference in the level of DDT usage by farmers within each region and suggests that there is lack of regulation or information on the use of these pesticides. The detection of high residue level of organochlorine pesticides (total DDT) in khat samples collected from different parts of Ethiopia shows that like other developing countries such pesticides are still in use. Currently, DDT is not permitted for agricultural use in Ethiopia. However, because of its easy accessible to the farmers from the depots meant for malarial control, it is illegally used on khat farms. Several reviews on the adverse health effects of chewing khat documented negative health impact of the active compounds in khat and in some cases in association with smoking (commonly used in khat chewing sessions) but did not account the role that may be contributed by pesticides (Al-Habori 2005). For instance, malathion has been reported to produce chromosomal aberrations and micronuclei in experimental animal studies, and immune system alterations at a sub-clinical level (Amer et al. 2002; Johnson et al. 2002).

Organochlorines have been shown to cause abnormalities in the reproduction and immune systems of birds and marine mammals and several abnormalities caused by organophosphorous have been reported in many organisms (Bonilla et al. 2008). The incidence of oral and oesophageal cancer among khat chewers (Al-Habori 2005) may also be in part attributed to the carcinogenic effects of organochlorine pesticides. It was observed that high number of local people in these regions suffer high incidences of cancer (*personnel observation* by the leading author), which is not surprising with such extremely high levels of pesticides contamination as well as the presence of other toxic compounds from khat and smoking. It is worth noting that the average time from last pesticide application to khat harvest was reported to be 7 days (Al-Hadrani and Thabet 2000) which is not sufficient to affect the pesticides load on the khat. The contamination of khat with pesticides and its potential adverse health effects should not be regarded as Ethiopian problem as many of the farming practices are shared in khat growing countries (Arabian Peninsula) and several European and Australasian countries has no restriction on the importation and the consumption of khat (Griffiths et al. 2010). Therefore such adverse effects could be extended to khat users in these communities.

The residue level of the studied pesticides in wheat from 3 different regions in Ethiopia is summarized in Table 3. Wheat samples from Adami Tulu had no detectable

Table 3 The observed pesticides residue level in wheat samples collected from Lole, Adami Tulu and Sinana area

Sample	Pesticides concentration ($\mu\text{g/Kg}$)					
	Diazinon	Malathion	Aldrin	<i>p,p'</i> -DDE	<i>o,p'</i> DDT	<i>p,p'</i> DDT
<i>Lole</i>						
Sample 1	66.5	ND	BQL	ND	ND	ND
Sample 2	120.5	ND	BQL	ND	ND	ND
Sample 3	143.0	ND	BQL	ND	ND	ND
Sample 4	173.0	ND	BQL	ND	ND	ND
<i>Sinana</i>						
Sample 1	51.0	ND	ND	ND	ND	ND
Sample 2	64.6	ND	BQL	ND	ND	ND
Sample 3	BQL	ND	ND	ND	ND	ND
Sample 4	378.0	ND	BQL	ND	ND	ND
Sample 5	103.9	ND	ND	ND	ND	ND
<i>Adami Tulu</i>						
Sample 1	ND	ND	ND	ND	BQL	ND
Sample 2	ND	ND	ND	ND	BQL	ND
Sample 3	ND	ND	ND	ND	BQL	ND
Sample 4	ND	ND	ND	ND	BQL	ND
Sample 5	ND	ND	ND	ND	BQL	ND

BQL Below quantification limit (the levels are as stated in footnote in table 2), *ND* Not detected

pesticides residues with the exception of *o,p'*-DDT which was detected in all wheat samples from that region at concentration below quantification limit ($>45.3 \mu\text{g/Kg}$).

Aldrin was detected below the quantification limit ($>48.0 \mu\text{g/Kg}$) in all wheat samples from Lole and in two samples from Sinana. Aldrin is not registered for use in Ethiopia for agriculture purposes and is not currently used for pest control. Detection of aldrin in samples from Lole and Sinana state farms indicate that it was illegally used for agricultural and pest control activities. This observation confirms an earlier findings in research conducted on the soil of Awash basin which indicated the presence of aldrin in soil (Westbom et al. 2008) and it's illegal availability and use. Neither DDT nor its metabolites were detected in any of the wheat samples from both Lole and Sinana state farms although wheat has low water and considerably high lipid content. Diazinon was detected in all the samples collected from both Lole and Sinana state farms. The mean diazinon residue level in the wheat samples from Lole and Sinana was 125.8 ± 45.0 and $149.4 \pm 154.1 \mu\text{g/Kg}$, respectively. The diazinon residue level determined in all the samples that tested positive was greater than the MRLs set for pesticides by EU- for cereals ($20 \mu\text{g/Kg}$) and this may be attributed to the common use of diazinon as a pesticide during the storage of wheat. Organophosphorus insecticides are widely used for protecting stored commodities from pests. While these compounds are mainly used to treat the stores, they are occasionally applied directly to the commodity. Direct application to commodities has attracted much attention

because residues in stored cereal grain generated through this practice pose tremendous health hazards to human (Uygun et al. 2008). The mean and range of diazinon concentrations in wheat samples collected from Lole and Sinana state farms were 125.8 (66.5 – $173.0 \mu\text{g/Kg}$) and $149.4 \mu\text{g/Kg}$ (51.0 – $378.0 \mu\text{g/Kg}$), respectively. These results highlight the variation in the dosage applied in different regions and within each region and demonstrate the lack or non compliance of standard protocols for pesticides use.

The results from the present study underline that pesticide residues are present in wheat and khat samples collected from the various sampling regions of Ethiopia. *p,p'*-DDT and *o,p'*-DDT in khat samples were observed at alarming levels (240–1,200 fold the EU MRL) while diazinon was detected in all the khat samples from BadaBuna above the MRL set for vegetables by EU. Diazinon levels in wheat samples from two regions out of the three regions under investigation were above the MRL set by EU for cereals. The level of pesticide residue observed in the samples investigated is of a magnitude that calls for a special attention to regulate the use and circulation of such chemicals. Routine monitoring of pesticides residue in different food items is necessary for the prevention, control and reduction of environmental pollution, as well as for legal decisions to minimize health risks.

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