PESTICIDE RESIDUE EVALUATION IN MAJOR STAPLE FOOD ITEMS OF ETHIOPIA USING THE QUECHERS METHOD: A CASE STUDY FROM THE JIMMA ZONE

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Abstract: Samples of maize, teff, red pepper, and coffee (green bean and coffee bean with pulp) were collected from a local market in the Jimma Zone, Ethiopia. Samples were analyzed for the occurrence of cypermethrin, permethrin, deltamethrin, chlorpyrifos ethyl, DTT and its metabolites, and endosulfan (α, β). In the analytical procedure, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction methodology with dispersive solid phase extraction clean up (d-SPE) technique was applied. Validation of the QuEChERS method was satisfactory. Recovery percentages of most pesticides were in the range of 70% to 120%, with good repeatability (%relative standard deviation < 20). The limit of detection and limit of quantification varied between 0.001 µg/g and 0.092 µg/g and between 0.002 µg/g and 0.307 µg/g, respectively. The main pesticides detected were DDT, endosulfan, cypermethrin, and permethrin. All of the pesticides analyzed were detected in red pepper and green coffee bean. Residues of DDT in coffee pulp significantly differed ($p < 0.01$) from other food items except for red pepper. The concentration of pesticides in the food items varied from 0.011 mg/kg to 1.115 mg/kg. All food items contained 1 or more pesticides. Two-thirds of the samples had residues below corresponding maximum residue limits, and the remaining one-third of samples were above the maximum residue limits. These results indicate the need for a good pesticide monitoring program to evaluate consumer risk for the Ethiopian people. *Environ Toxicol Chem* 2014;33:1294–1302. © 2014 SETAC

Keywords: Pesticide residues   QuEChERS method   Staple food   Teff   Red pepper

INTRODUCTION

Pesticides are applied worldwide to control a variety of pests affecting agricultural crops, disease vectors, and household pests. Widespread application of pesticides may affect human and environment health as a result of residues that remain in different environmental matrices and consumer items such as food, water, and air [1]. Pesticide residues in food form an important route of pesticide exposure to humans. In areas where there is widespread use of pesticides, food safety has became a top public health concern [2]. Pesticide residues can be found in different food items such as dairy products, cereals, fruits, vegetables, and cash crops such as coffee [3–9]. In Nigeria, for example, there is evidence of excessive agricultural use of pesticides on cereals such as maize [10]. One study found that approximately 96% of the maize samples contained residues of 1 or more organochlorine pesticides (OCPs), and some samples contained OCPs above corresponding maximum residue limits [11]. In Ghana, organophosphate, organochlorine, and pyrethroid pesticides have been detected in vegetables and fruits [9], and OCP contamination of cow's milk has been shown in Giza, Egypt [6]. Obsolete pesticides have been documented as one of the major problems in Africa, particularly in Ethiopia [12]. The OCPs are often detected in different food items mainly as a result of environmental contamination from crop application and leaching from dumped obsolete stocks [13]. In Ethiopia, indoor and outdoor application of pesticides is a daily practice to increase productivity and to protect different food items from various pests before and after harvesting. In addition, the Ethiopian Federal Ministry of Health uses indoor residual sprays such as DDT and dieldrin to protect people against mosquito-transmitted diseases [14]. These activities could potentially contaminate different food items produced in Ethiopia, which in turn may impact public health.

The most common food items produced in Ethiopia are maize, teff, sorghum, millet, dairy products, oilseeds, and coffee [15]. Maize is a major crop, and thus a major staple food item for millions of Ethiopians, particularly in the Jimma Zone of southwest Ethiopia [16]. According to Gebremedhin et al. [15], maize is the most important crop in Ethiopia for household consumption (accounting for 65%), followed by teff (37%), and maize covers approximately 47% of the cultivated area in the country. To increase the yield, different hybrid maize varieties are used, and the commonly cropped hybrid variety can easily be affected by a variety of insects in the field and during storage. As a result, different pesticides are applied, and their residue may remain on the crop [17].

Teff (*Eragrostis tef*) is primarily grown in Ethiopia for human consumption of its grain and has been used for centuries. It is the other principal food ingredient in the Ethiopian diet. Teff is usually made into *injera*, a large flat bread or pancake, which is eaten alone or with any kind of meat, vegetables, or sauces. In addition, teff flour is used for the preparation of porridge and kita (nonfermented bread) [18].

Coffee is a popular cash crop. Ethiopia is Africa's biggest coffee producer and exporter (followed by Ivory Coast and Uganda), and it provides more than 4% of the world's coffee [19]. Coffee is also the most widely consumed stimulant beverage in the country. Approximately 50% of the total coffee produced is consumed locally. The annual per capita consumption of coffee in Ethiopia is approximately 2.4 mg/kg, which is comparable to the consumption level of the leading coffee-consuming countries (A. Zeru, 2006, Master's thesis, Addis Ababa University School of Graduate Studies, Addis Ababa, Ethiopia).

Red pepper is another ancient vegetable cultivated in Ethiopia [20] and is used widely in Ethiopian cuisine. Fine red pepper powder is an indispensible flavoring and coloring ingredient in a common traditional sauce, *wat* [21]. A study done in the Jimma Zone indicates that red pepper production is the largest in the surrounding areas, and 50% of the sales goes directly to local consumers [22]. Red pepper is the major spice produced in more than 9 districts of the Jimma Zone [21].

The agricultural office in the Jimma Zone has said that different types of pesticides are necessary to guarantee high productivity (G. Belay, Yebu Agricultural Office, Jimma, Jimma Zone, Ethioiopia, Personal Communication). Pyrethroids (permethrin, cypermethrin, and deltamethrin) and organophosphates (chlorpyrifos ethyl, malathion, diazinon) are the major pesticides applied on agricultural fields,and organochlorine pesticide (endosulfan and DDT) environmental contamination results from indoor residual spraying, contaminated surfaces at storage places, and the presence of obsolete pesticides in areas nearby (M. Temam, Zonal Agricultural Office, Jimma, Jimma Zone, Ethioiopia, Personal Communication). The present study hypothesized that application of these pesticides and historical use of some of the persistent pesticides such as DDT result in traces of these pesticides in food items. Pesticide residues can be found in all environmental compartments, but the highest risk for consumers is through consumption of residues in food [23].

In the accessible scientific literature of Ethiopia, there is almost no information regarding pesticide residue levels in maize, teff, red pepper, coffee, and other food items except for wheat, khat [24], and cow and human milk [5]. The present study presents data on the level of pesticide residues in selected staple food items sold in the local market of the Jimma Zone. The study also compares the residues in food items with accepted international food safety limits.

Determination of pesticide residues in different food items using the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) has been documented to give a better recovery compared with classical techniques of liquid–liquid extraction [25]. The classical extraction methods often require a large sample size because of multistage procedures and require more clean-up steps. As a result, classical methods are time consuming, labor intensive, and expensive, and can produce a considerable amount of waste in the environment [26]. Thus, analytical chemists now prefer to use the QuEChERS method with a dispersive solid-phase extraction (d-SPE) clean-up; it is streamlined and effective for analysis of diverse residues in food matrices [26]. The QuEChERS method has the following advantages: high recovery, high sample throughput, low solvent and glassware usage (no chlorinated solvents), less labor and bench space, lower reagent costs, ruggedness, and low worker exposure [27,28]. The aim of the present study was to evaluate pesticide residues in selected staple food items of Ethiopia using the QuEChERS method as the extraction and clean-up technique.

MATERIALS AND METHODS

Sampling

A total of 42 samples from staple food items (8 maize samples, 9 teff samples, 10 red pepper samples, 7 green coffee bean samples, and 8 coffee samples with pulp) were collected from the local market in the Jimma Zone. During sample collection, the market retailers were interviewed about the origin of the crops and from how many farmers they bought these crops. Most of the retailers said that they bought 1 type of food item from at least 5 farmers and pooled (not statistically) each food item before bringing it to the market for sale. Based on this experience, a retailer was chosen as a crop sample source only if the target crop had been pooled from at least 5 farmers in a similar crop production location (district). Locations where staple food items were commonly produced included Agaro, Bonga, Dedo, Ela, Goro, Kersa, Limu, Nanda, Seka, Sekoru, Shebe, Tocha, Woliso, Wonchi, Yebu, and Yem (Figure 1). Each 250-g sample was sealed, labeled with a unique sample identity, and placed in a clean hard paper envelope within polyethylene plastic bags. Then the samples were transported to the Laboratory of Crop Protection Chemistry at Ghent University, Belgium, and frozen at –20 °C until analysis.
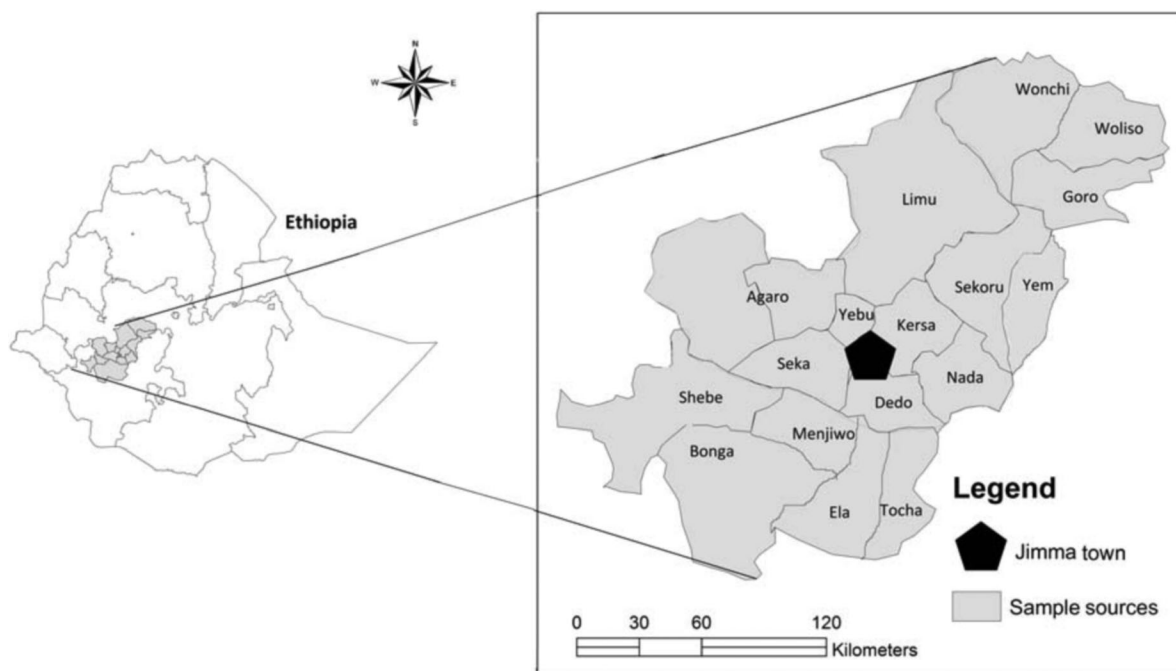

Figure 1. Map of study area indicating sample sources and the Jimma town where the samples were collected.

Sample preparation

A 250-g sample (each) of maize, teff, red pepper, and coffee was first ground and then homogenized using a household mill equipped with a stainless steel knife (Fritel Quality grinder OZX48-6 cups) following the procedure of Dasika et al. [29]. After each sample was ground, the grinder was washed to avoid cross-contamination. The ground and homogenized samples were packed in plastic bags and stored at –20 °C until extraction.

Reagents and materials

Analytical grade acetonitrile was supplied by VWR-PROLABO, and high-performance liquid chromatography grade n-hexane and acetone were obtained from ALLtech. Thermo Fisher Scientific supplied magnesium sulfate ($MgSO_4$) to remove water from organic solvent, sodium acetate (NaAc) to absorb remaining water content in samples, 50-mL polypropylene centrifuge tubes, 15-mL d-SPE tubes packed with primary and secondary amines (PSAs) for the removal of organic acids and polar pigments among other compounds, and octadecyl (C18) to remove lipids and sterols from the matrix. Pesticide standards, dichlorodiphenyltrichloroethane (DDT) (*p,p'*-dichlorodiphenyldichlorethylene [DDE], *p,p'*-dichlorodiphenyldichlorethane (DDD), *o,p'*-DDT, and *p,p'*-DDT), endosulfan (α and β), cypermethrin, permethrin, deltamethrin, and chlorpyrifos ethyl of highest analytical purity were obtained from Supelco and delivered by Sigma-Aldrich Logistics. The respective purities of reagents, materials, and pesticide standards are presented in Table 1.

Analytical equipment

All pesticide residues were determined by gas–liquid chromatography with an electron capture detector (GC-ECD; Agilent Technologies 6890N) and an autosampler. An HP-5 capillary column (30 m × 0.25 mm inner diameter; 0.25-μm film thickness) coated with 5% phenyl methyl siloxane (model 19091J-433; Agilent) was used in combination with the following oven temperature program: initial temperature of 80 °C, ramped at 30 °C min⁻¹ to 180 °C, ramped at 3 °C min⁻¹ to 205 °C, held for 4 min, ramped at 20 °C min⁻¹ to 290 °C, held for 8 min, ramped at 50 °C min⁻¹ to 325 °C. For deltamethrin, the oven temperature was maintained initially at 130 °C, held for 1 min, ramped at 30 °C min⁻¹ to 280 °C, held for 16 min, ramped at 50 °C min⁻¹ to 325 °C, and held for 3 min. The total GC run time was 27.92 min. Helium (99.9999% purity) was used as a carrier gas at a flow rate of 20 mL min⁻¹ and nitrogen as a makeup gas at a flow rate of 60 mL min⁻¹. An aliquot of 1 μL

Table 1. Pesticide standards, reagents, and QuEChERS materials with their respective analytical purities

Pesticide standard	Purity (%)	Reagent	Purity (%)
p,p'-DDE	99.9	Acetonitrile	99.9
p,p'-DDD	99.3	n-Hexane	95
o,p'-DDT	100	Acetone	99
p,p'-DDT	99	Glacial acetic acid	98.5
Endosulfan α	98.5	$MgSO_4$	98
Endosulfan β	98	NaAC	99
Deltamethrin	99	PSA	100
Permethrin	98		
Cypermethrin	98		
Chlorpyrifos ethyl	99.5		

QuEChERS = quick, easy, cheap, effective, rugged, and safe; DDE = dichlorodiphenyldichlorethylene; DDD = dichlorodiphenyldichlorethane; DDT = dichlorodiphenyltrichloroethane; $MgSO_4$ = magnesium sulfate; NaAC = sodium acetate; PSA = primary and secondary amine.

was injected in split mode at a split ratio of 50:1 and injection temperature of 280 °C. The pesticide residues were detected with μ-ECD operating at a temperature of 300 °C. The pesticide residues in each food sample were analyzed in triplicate, and the mean concentration was computed accordingly.

Analytical method validations

The limit of detection (LOD) and the limit of quantification (LOQ) were determined by preparing matrix spikes at a low level near the expected detection limit. The LOD and LOQ in the present study were determined by spiking a concentration of 0.04 mg/L for chlorpyrifos ethyl and cypermethrin; 0.1 mg/L for permethrin, deltamethrin, and DDT and its metabolites (*p,p'*-DDE, *p,p'*-DDD, and *o,p'*-DDT); and 0.01 mg/L for endosulfan (α and β) in maize, green coffee bean, teff, and red pepper in 6 replicates. The LOD and LOQ were calculated by multiplying the standard deviation from the replicates by 3 and 10, respectively [30]. The accuracy and precision of the method (i.e., recovery percentage and repeatability, respectively), expressed in terms of relative standard deviation, were determined with recovery experiments by spiking pesticide standards into a laboratory blank sample of crops in 6 replicates with the same concentration of each pesticide. Afterward, the relative standard deviation was calculated by dividing the standard deviation by the average concentration.

The linearity was determined by preparing a stock solution of pure standards of the pesticides studied and diluting them to produce different concentrations. The standard solutions of the pesticides were run on GC-ECD under the set chromatographic conditions to produce 5-point calibrations ranking from 0.01 mg/L to 1 mg/L.

The recovery tests were done by spiking a mixture of 10 pesticides including isomers (endosulfan α and β) and metabolic products (*p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDT, and *p,p'*-DDT). A pesticide standard was spiked into laboratory blank samples of maize, green coffee bean, teff, and red pepper to give 0.25 μg/g, and recovery was based on 4 replicates. The spiked samples were left for 1 h before extraction to allow the pesticides to partition into the matrices [9].

QuEChERS method

Extraction and clean-up of the spiked samples and blank samples from each matrix were performed using the modified QuEChERS procedure with the d-SPE clean-up method. The procedures were based on Association of Analytical Communities official method 2007.01 [31] with slight modifications. This method gives high-quality results for many pesticides [29]. The procedure for spiking and extraction is as follows: 1) 10 g of comminuted and homogenized blank sample of maize, green coffee bean, teff, and red pepper were weighed in 50-mL centrifuge tubes on an analytical balance (Sartorius); 2) 10 mL of deionized water was added; 3) blank samples were spiked with 25 μL of each pesticide standard in each matrix in 4 replicates; 4) 15 mL of acetonitrile containing 1% glacial acetic acid (v/v) in each sample was added using a solvent dispenser; 5) the tube was tightly capped and shaken gently for 1 min to facilitate contact between the solvent and the sample; 6) 6 g anhydrous $MgSO_4$ and 1.5 g NaAc were added, and the sample was shaken by hand vigorously for 5 min to increase sample throughput [28]; 7) the sample was centrifuged at 2016 *g* for 5 min; 8) for clean-up, the upper 8 mL to 10 mL were put into a d-SPE tube containing 300 mg PSA, 900 mg $MgSO_4$, and 150 mg C18 and shaken by hand for 30 s, and then step 6 was repeated; 9) a 5-mL aliquot of cleaned extract was then taken and evaporated to dryness using a

rotary evaporator (N18673 Rotavapor; Buchi) at a temperature of 40 °C; 10) the cleaned extract was reconstituted with 2 mL n-hexane:acetone (9:1) for solvent exchange; and 11) the extract was then put into an autosampler vial for GC analysis.

Analysis of samples

The extraction and clean-up of teff, maize, red pepper, green coffee bean, coffee pulp, and coffee bean (after removal of the pulp) samples were done following procedures similar to those described for the above recovery test, except for spiking. From 250 g of ground and homogenized samples, a representative 10-g subsample was weighed in a 50-mL centrifuge tube on an analytical balance. Then 10 mL of deionized water was added to the sample and shaken for 1 min. Then steps 3–10 described in the *QuEChERS method* section were followed. The cleaning procedure between samples was done by injecting a blank (n-hexane), pesticide standards for calibration, and n-hexane again to ensure data quality. The sample equivalent (mg/mL) extract was calculated based on the formula suggested by Schenck and Howard-King [32]

$$Y = a/b \times x/z$$

where Y is grams of sample equivalent per milliliter of extract, a is the amount of sample analyzed (g), b is the volume of solvent added to extract the sample (mL), x is the amount of the cleaned extract taken after evaporation until dryness (mL), and z is the amount of hexane:acetone (9:1, v/v) added for solvent exchange (mL).

Data analysis

The Wilcoxon signed rank sum test was performed to test for significant differences of pesticide residue between the food items. Box and whisker plots were used to compare the distribution of the pesticides analyzed in the different food samples. In the statistical analysis, $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Method validation results

Linearity of the standards. The calibration curves obtained for a mixture of 10 pesticide standards, including isomers and degradation products over a concentration range of 0.04 mg/L to 1 mg/L (chlorpyrifos ethyl and cypermethrin), 0.05 mg/L to 1 mg/L (deltamethrin), 0.01 mg/L to 0.2 mg/L (endosulfan α and

β), and 0.1 mg/L to 1 mg/L (p,p′-DDE, p,p′-DDD, o,p′-DDT, and p,p′-DDT) showed a correlation factor (r^2) higher than 0.9955.

Recovery studies. The average recoveries of pesticides analyzed from the food matrices in replicate tests are shown in Table 2. The results revealed that the QuEChERS method is sufficient to achieve satisfactory pesticide recoveries in the matrices evaluated. The average recoveries of DDT and its metabolites, permethrin, and chlorpyrifos ethyl were in the acceptable range (70–120%) [25]. The average recoveries of cypermethrin were above 120% in all food matrices of the replicate tests. According to the Swedish National Food Administration, if the recoveries of pesticides are consistently high or low in the replicate tests, this outcome is acceptable [25]. The most apolar pesticides tested (endosulfan α and β) showed consistently low recoveries (<60%) but with good repeatability (relative standard deviation < 10%). This low recovery may be because of losses during the extraction and clean-up steps with C18 and PSA. Among the food matrices, the recovery of endosulfan was lower in green coffee bean: 50.1% and 34.3%, for α and β isomers, respectively. Pizzutti et al. [33] reported that the modified QuEChERS method is known to obtain lower recoveries for apolar pesticides like endosulfan extracted from fattier matrices such green coffee beans, which has a fat content of 7% to 17%.

Precision, LOD, and LOQ. The LOD and LOQ varied from 0.001 μg/g to 0.092 μg/g and 0.002 μg/g to 0.307 μg/g (Table 2), respectively, for the pesticides evaluated. Both the LOD and LOQ were below the lowest standard concentration, which indicates that the analytical method is able to detect and quantify still lower concentrations from the food matrices [25]. The relative standard deviation is the measure of agreement or consistency of analyte concentrations to each other when the analyses are performed using identical conditions, that is, the same method, same sample, same operator, and same laboratory conditions over a short period of time [34]. The relative standard deviation for all the pesticides studied were below 11% which is in line with the commonly accepted level (relative standard deviation < 20%) [35]. The result of the present study is in consistent with the study done in Portugal on multi-pesticides residue analysis using acetonitrile extraction, with a relative standard deviation < 20% for all the pesticides detected [36].

Pesticide residue in tested food samples. After validation of the QuEChERS method, the pesticide residues in maize, teff, green coffee bean, coffee pulp, and coffee bean (after removal of pulp) were determined. The results revealed that each food item

Table 2. Method validation results including recovery, LOD, and LOQ in food matrices evaluated (in μg/g)[a]

Pesticide	Spiked conc.	Maize			Green coffee bean			Red pepper			Teff		
		Recovery (%)	LOD	LOQ	Recovery (%)	LOD	LOQ	Recovery (%)	LOD	LOQ	Recovery (%)	LOD	LOQ
Chlorpyrifos ethyl	0.2	120.4 (1.4)	0.009	0.031	101.4 (1.0)	0.006	0.021	98.9 (1.6)	0.012	0.041	99.3 (1.6)	0.009	0.031
Cypermethrin	0.2	143.2 (9.5)	0.059	0.197	121.7 (9.9)	0.032	0.107	132.2 (9.8)	0.061	0.202	135.1 (10.3)	0.062	0.207
Permethrin	0.5	113.5 (4.9)	0.065	0.218	106.3 (8.8)	0.092	0.307	102.9 (4.7)	0.063	0.209	87.0 (5.7)	0.063	0.209
Deltamethrin	0.5	123.3 (1.3)	0.022	0.075	135 (0.8)	0.016	0.055	108.9 (1.3)	0.018	0.06	96.9 (1.0)	0.015	0.05
p,p′-DDE	0.5	77.2 (1.0)	0.012	0.041	73 (1.4)	0.01	0.033	80.3 (1.2)	0.016	0.053	78.4 (1.1)	0.016	0.053
p,p′-DDD	0.5	102 (2)	0.028	0.092	80.1(1.7)	0.022	0.072	93.5 (1.5)	0.023	0.076	102.6 (1.5)	0.023	0.076
o,p′-DDT	0.5	86 (1.4)	0.015	0.051	86.7 (1.3)	0.015	0.051	89.4 (3.3)	0.045	0.151	94.1 (2.4)	0.036	0.121
p,p′-DDT	0.5	94.2 (1.6)	0.019	0.064	99 (1.8)	0.02	0.066	104.4 (2.5)	0.03	0.1	101.5 (2.2)	0.03	0.1
Endosulfan α	0.05	57.4 (1.2)	0.001	0.002	50.1 (1.1)	0.001	0.004	50.6 (3.3)	0.004	0.014	52.7 (4.3)	0.004	0.014
Endosulfan β	0.05	46.5 (1.7)	0.001	0.003	34.3(2.7)	0.001	0.004	44.1 (3.4)	0.005	0.016	38.1 (9)	0.004	0.014

[a]Values in parentheses are relative standard deviation (%).
LOD = limit of detection; LOQ = limit of quantification; DDE = dichlorodiphenyldichlorethylene; DDD = dichlorodiphenyldichlorethane; DDT = dichlorodiphenyltrichloroethane.
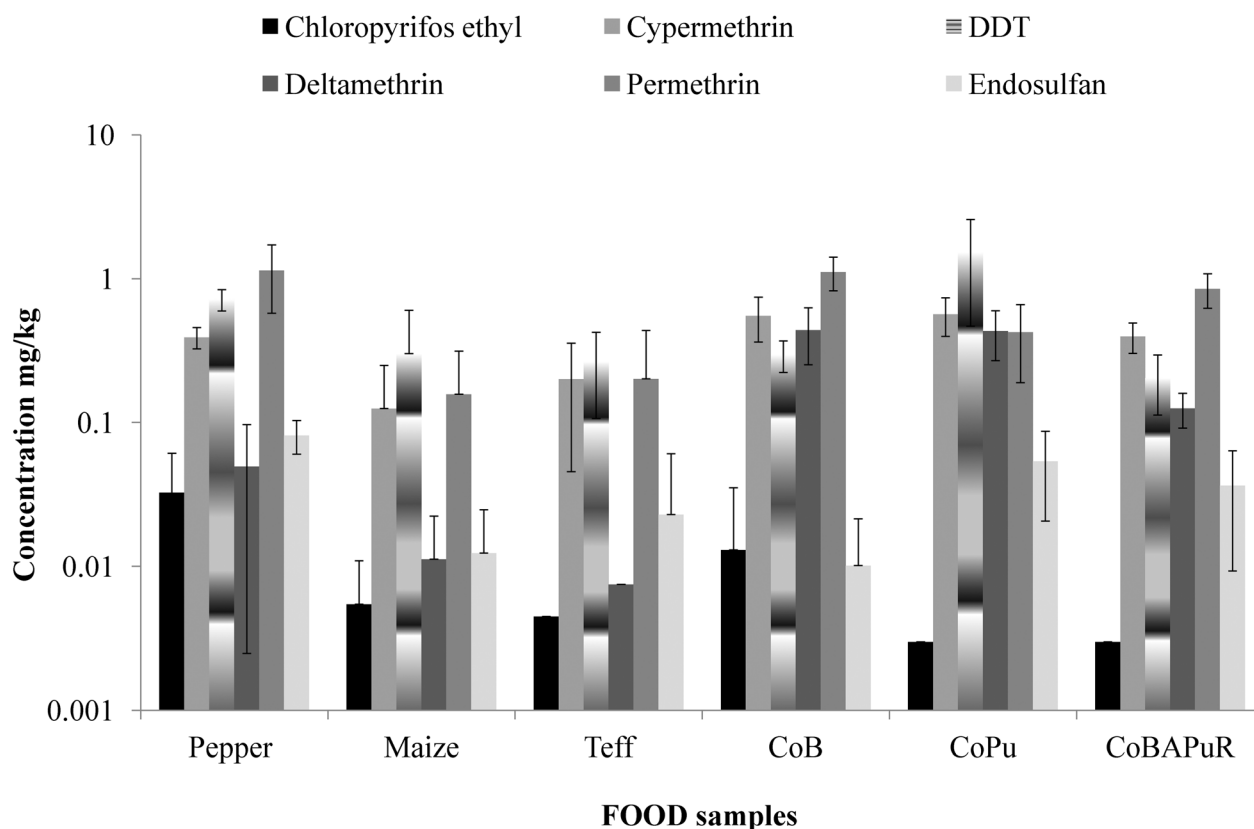
Figure 2. Average concentration (mg/kg) of pesticides in evaluated food samples. Error bars indicate the standard deviation. Copu = coffee pulp; CoB = green coffee bean; CoBAPuR = coffee bean after removal of the coffee pulp.

Table 3. Residue levels of pesticides in some African countries compared with Ethiopia[a]

Country	Pesticide	Concentration (mg/kg)	Food item	Reference
Ghana	p,p'-DDE	0.004–0.0.041	Watermelon, cabbage, mango, and banana	Bempah et al.[9]
	p,p'-DDT	0.004–0.038	Carrot, watermelon, onion, and banana	
	Chlorpyrifos	0.003–0.055	Watermelon, tomato, pineapple, or onion	
	Cypermethrin	0.008–0.035	Mango, cucumber, papaya, or tomato	
	Permethrin	0.004–0.041	Cucumber, pear, cabbage, and pineapple	
	Deltamethrin	0.004–0.044	Carrot, pear, onion, and pineapple	
Nigeria	p,p'-DDE	0.00–0.175	Chili pepper and tomato	Benson and Olufunke [38]
	p,p'-DDD	<LOD–0.172	Chili pepper and tomato	
	p,p'-DDT	0.036–0.099	Tomato and chili pepper	
Togo	p,p'-DDE	0.0045–0.0072	Cowpea and maize	Mawussi et al. [39]
	p,p'-DDD	<LOD–0.0051	Maize and cowpea	
	o,p'-DDT	0.002–0.055	Maize and cowpea	
	p,p'-DDT	0.011–0.023	Cowpea and maize	
	Endosulfan α	0.034–0.10	Maize and cowpea	
	Endosulfan β	0.065–0.09	Maize and cowpea	
South Africa	Chlorpyrifos	0.01–0.05	Wheat	Dalvie and London [40]
	Cypermethrin	0.008–0.019	Wheat	
	Permethrin	0.014–0.448	Wheat	
Ethiopia	p,p'-DDE	0.00–0.086	CoBPuR COB, teff, red pepper, and maize	Present study
	p,p'-DDD	0.049–0.128	Maize, CoBPuR, teff, COB, Red pepper and CoPu	
	o,p'-DDT	0.085–0.193	COB, CoBPuR, CoPu, teff, maize, and red pepper	
	p,p'-DDT	0.099–0.461	CoBPuR, teff, maize, CoB, red pepper, and CoPu	
	Chlorpyrifos	0.011–0.063	CoBPuR or CoPu, teff, maize, red pepper, and CoB	
	Endosulfan α	0.0042–0.0332	CoBPuR, teff, maize, CoB, CoPu, and red pepper	
	Endosulfan β	0.002–0.063	CoBPuR, maize, CoB, teff, Red pepper, and CoPu	
	Cypermethrin	0.156–0.553	maize, CoBPuR, teff, CoPu,red pepper, and CoB	
	Permethrin	0.157–1.15	maize, teff, CoPu, CBPR, Red pepper, and CoB	
	Deltamethrin	0.069–0.157	Red pepper, CoBPuR, CoPu, and CoB	

[a]The food items are listed in increasing order of pesticide residues.
DDE = dichlorodiphenyldichlorethylene; DDT = dichlorodiphenyltrichloroethane; DDD = dichlorodiphenyldichlorethane; CoB = green coffee bean; CoPu = coffee pulp; CoBPuR = coffee bean after removal of the pulp; LOD = limit of detection.

analyzed had at least 1 pesticide (Figure 2). Specifically, DDT, endosulfan, cypermethrin, and permethrin were the most abundantly detected pesticides. The red pepper and green coffee bean samples contained all the pesticides evaluated at varying concentrations. Pesticide residues varied from 0.0321 mg/kg to 1.148 mg/kg in red pepper, 0.011 mg/kg to 0.301 mg/kg in maize, 0.014 mg/kg to 0.351 mg/kg in teff, 0.016 mg/kg to 1.115 mg/kg in green coffee bean, 0.077 mg/kg to 1.519 mg/kg in coffee pulp, and 0.037 mg/kg to 0.619 mg/kg in coffee bean (after removal of pulp). In several samples, DDT and the metabolites p,p'-DDE (19.04%), p,p'-DDD (92.9%), o,p'-DDT (66.67%), and p,p'-DDT (100%) were detected.

In the coffee samples analyzed, the maximum concentration was found in coffee pulp (1.519 mg/kg); a lower concentration of DDT was found in coffee bean after removal of the pulp (0.244 mg/kg) followed by green coffee bean (0.301 mg/kg). The lower residue detected in green coffee bean might be the result of the rich nutrient content available, which may promote the growth of microorganisms that are able to degrade the pesticides [37]. In addition, the maximum concentration in coffee pulp may be the result of the deposition of these contact pesticides on the surface of the crop. Compared with recent studies in some African countries (Ghana [9], Nigeria [38], Togo [39], and South Africa [40]; Table 3), the staple food items
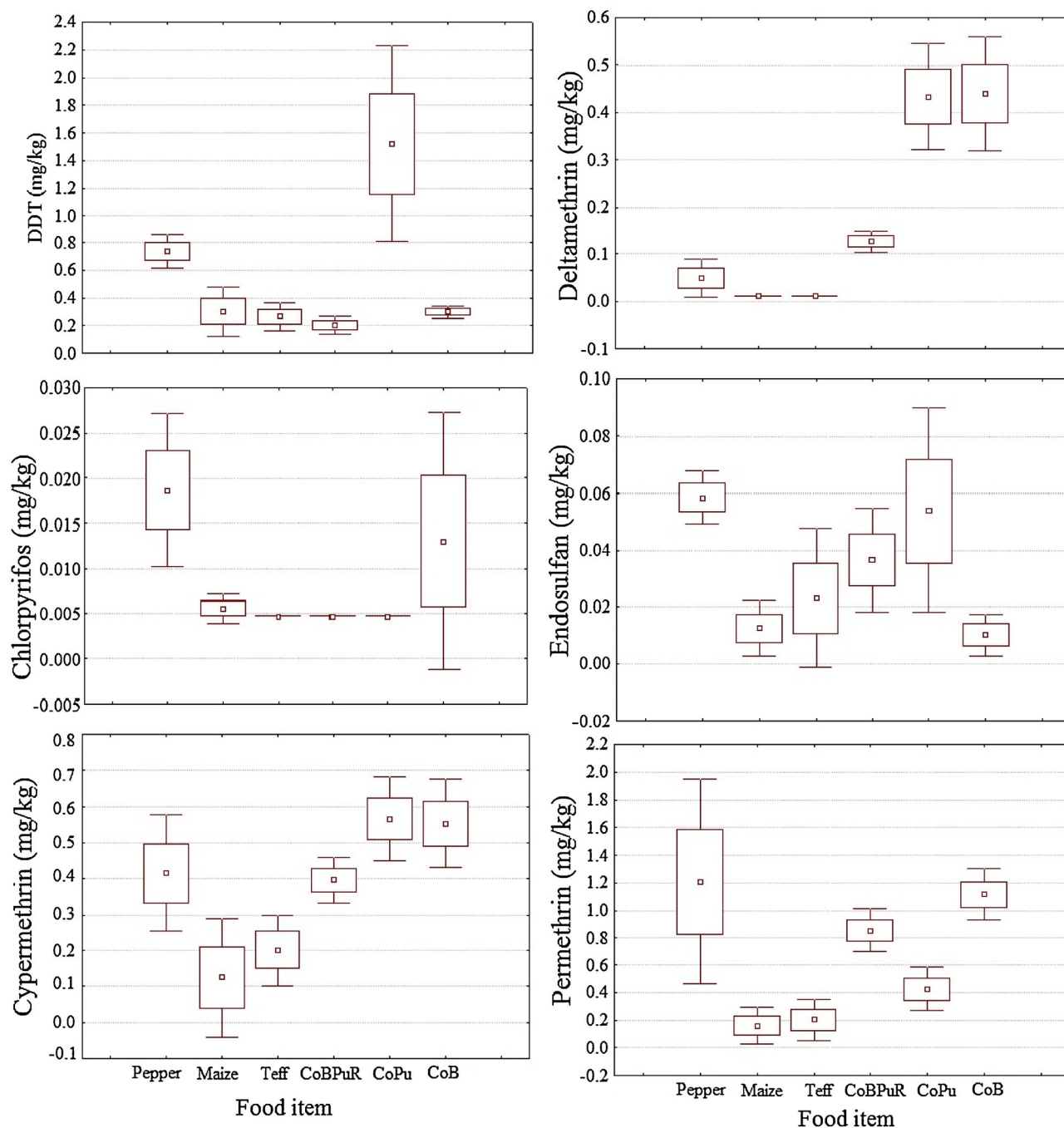

Figure 3. Concentration distribution of pesticide residues in evaluated food items. The small square within each box represents the mean; the box represents the mean ± standard error; and upper and lower whiskers represent 95% confidence intervals. Copu = coffee pulp; CoB = green coffee bean; CoBAPuR = coffee bean after removal of the coffee pulp.

in the Jimma Zone contained higher concentrations of all pesticide residues except for some Nigerian food items. This might be explained by the extensive application of pesticides to obtain higher production, insufficient knowledge of how to apply the correct rate, and poor equipment available to Ethiopian farmers for spraying the pesticides [12]. In addition, higher residues could result from historical use and contamination, particularly by those compounds demonstrating environmental persistence and by accumulation of obsolete pesticides [12]. In Nigeria, Tijani and Nurudeen [10] have explained that the higher pesticide residues in the food items they evaluated were the result of the excessive use of OCPs.

Although DDT is officially banned for agricultural application, contamination of food still occurs. This contamination might be explained by indoor spraying of DDT for malaria prevention and by illegal use from obsolete pesticide stocks [12]. Records indicate that the most dangerous pesticides such as DDT, aldrin, heptachlor, pyrimifos methyl, and fenitrothion are the main components in obsolete stocks dumped at more than 1000 sites in Ethiopia [41]. Endosulfan was detected in 61.9% of the food items but at a lower concentration than the other pesticides studied. Compared with a study done in Togo [39] (Table 3), the residue levels in the present study were 3 times and 2 times lower for endosulfan α and β, respectively. This might be because of less contamination of the food items by endosulfan in the study area or low recovery of this pesticide from the matrices evaluated. Post hoc analyses compared the distribution of pesticide residues between crop samples (illustrated by box and whisker plots; Figure 3). For this statistical comparison, the nondetects were included as being half the LOD [42]. Significantly higher concentrations of cypermethrin were observed in green coffee bean and coffee pulp relative to maize and teff ($p < 0.01$). This indicates a high variation in the use of chemicals among food items. No significant differences were observed for the concentration of chlorpyrifos ethyl between food items evaluated. The concentration of DDT in coffee pulp significantly differed ($p < 0.01$) from other food items except for red pepper. This might be because of illegal use of DDT in coffee and the persistent (lipophilic) nature of DDT [9]. In general, lower concentrations of pesticides were observed in samples of maize and teff. This may be because of less usage of the pesticides evaluated in these food items in the area studied.

Comparison of pesticide residue level with international maximum residue limits. Pesticide residues in samples of red pepper, maize, green coffee bean, teff, and coffee bean after removal of pulp were compared with corresponding international maximum residue limits (MRLs; Table 4). In all samples analyzed, two-thirds had residues below the MRLs, whereas one-third had a residue above the MRL set by the Codex Alimentarius [43]. Cypermethrin most frequently exceeded the corresponding MRL (11.9%), followed by permethrin (9.52%), DDT (4.76%), and endosulfan, chlorpyrifos ethyl, and deltamethrin (2.38% each).

The value for DDT, which was expressed as the sum of its metabolites (p,p'-DDE, p,p'-DDD, o,p'-DDT, and p,p'-DDT [43]), was above the corresponding MRL of the maize and teff cereals. Among the metabolites, p,p'-DDE contributed less to the total DDT residue load. In contrast, p,p'-DDT, which was found in all the samples analyzed, contributed more to the total DDT residue load. This might indicate recent use of DDT in the study area. The recent use of DDT was also observed by the European Food Safety Association [44]. Pesticides found in food items higher than the corresponding MRLs may indicate illegal use of these pesticides and may potentially affect consumers' health. According to Bempah et al. [9], even low exposure to pesticide residues may accumulate and pose a risk to consumers. Consequently, under the framework of chronic and acute food safety risk assessments, pesticide residue data below the MRL are also considered [45].

For the pesticides evaluated, no MRL was found for teff and coffee pulp, perhaps because of the unfamiliarity of these food items worldwide. However, in the present study, the MRL for grain was considered, to evaluate the residue in teff. Because teff is the major grain consumed in Ethiopia and is a principal staple food for almost all Ethiopians [18], the MRL for different pesticides should be established. As the consumption of coffee pulp becomes more and more important in rural Ethiopia [46], monitoring of this coffee component should also be a focus for consumer safety. The concentrations of all pesticides decreased in coffee bean after pulp removal except for permethrin. This might be because of the deposition of most pesticides on the surface of the crop and the mode of action (contact).

CONCLUSIONS

The QuEChERS method with d-SPE clean-up was effective for analysis of the pesticides evaluated in several Ethiopian food items. All samples collected from the local market in the Jimma Zone contained 1 or more pesticides. Approximately one-third of the samples had pesticide residues above MRLs set by the Codex Alimentarius, whereas two-thirds were below the MRLs. Red pepper and green coffee bean samples contained all

Table 4. Comparison of pesticide residues (in mg/kg) in food samples with corresponding MRLs set by the Codex Alimentarius[a]

Pesticide	Red pepper		Maize		Teff		CoB		CoBPuR	CoPu[c]
	Residue	MRL	Residue	MRL	Residue	MRL[b]	Residue	MRL		
Chloropyrifos ethyl	0.032	2	0.011	0.05	ND	0.05	0.063[a]	0.05	ND	ND
Cypermethrin	0.390[a]	0.1	0.156[a]	0.05	0.351[a]	0.03	0.553[a]	0.05	0.267[a]	0.433
DDT	0.734	NA	0.301[a]	0.1	0.296[a]	0.1	0.308	2.0	0.244	1.519
Deltamethrin	0.069[a]	0.03	ND	2.0	ND	2	0.440	1.0	0.102	0.356
Permethrin	1.148[a]	0.01	0.157	2.0	0.282[a]	0.05	1.115[a]	0.05	0.619[a]	0.495
Endosulfan	0.0599	5.0	0.012	NA	0.014[a]	0.01	0.016	0.2	0.037	0.077

[a]Values are above the MRL.
[b]The MRL of grains was adapted for teff.
[c]Coffee pulp has no MRL value.
MRLs = maximum residue limits; NA = not available; ND = not detected; CoB = green coffee bean; CoPu = coffee pulp; CoBPuR = coffee bean after removal of the pulp.

pesticides evaluated. Some banned pesticides and those not authorized for use in cereals, vegetables, and coffee, such as organochlorines (e.g., DDT and endosulfan), were also detected. Recent use of DDT was observed in the study area. The establishment of MRLs for teff and coffee pulp for pesticides used in the cultivation of these crops is necessary to safeguard consumer health. More monitoring of pesticide residues in other food items will result in a better risk characterization and food safety assurance.

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