Pesticide residues determination in food premises using QuECHERS method in bench-maji zone, southwest Ethiopia.

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Abstract

Pesticides are very important risk factors in human life causing chronic human health effects. Pesticides are commonly used across the globe to enhance human endeavors. In Ethiopia, pesticides are widely used by local farmers and governmental organizations for pests control purpose. Pesticide residues in food items have been a concern to the consumers and environment they live in. Therefore, this study was aimed to determine the amount of organo-chlorine and organo-phosphate pesticide residues in cereal crops in Bench-Maji Zone, Ethiopia. Cross sectional laboratory based study design was employed to determine the amount of pesticide residues. The samples were extracted using a Quick, Easy, Cheap, Effective, Rugged and Safe extraction and clean up method. Finally, the extracted samples were injected into Gas chromatography tandem Mass Spectrometer and the types, and concentrations of pesticide residues were analyzed.

Keywords: Pesticides, Residues, Cereal crops, Bench-Maji, Ethiopia.

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Introduction

Pesticides are poisonous chemicals intended for preventing, destroying or controlling pests during the production, processing, transporting and marketing of food. This includes vectors of human and animal diseases, unwanted species of plants or animals. The use of chemicals in modern farming practices is viewed as an integral part of the success of the agricultural industry. However, most of the pesticides applied to agricultural lands may affect non-target organisms and contaminate soil and water media [1]. In recent years there have been an increasing concern that pesticides constitute a risk to the general population through residues in food supply.

Most pesticides used in agriculture today are synthetic organic chemicals that act by interfering with a vital metabolic process in the organisms to which they are targeted. The public health effects of pesticides have long been known and the undesired effects of chemical pesticides have been recognized as a serious public health concern during the past decades [2]. According to a market survey report, approximately 5,684 million pounds of pesticides (active ingredients) are applied annually throughout the world. Many of these chemicals are mutagenic and linked to the development of cancer or may lead to birth defects.

Genotoxic effects of Malathion, Cypermethrin and Carbosulphan in chromosomal aberration and sperm abnormality in mice as potential germ cell mutagens. The health effects of pesticides that can be divided into acute poisoning and chronic effects. Acute pesticide poisoning is any illness or health threats appearing shortly after a single or multiple doses of pesticide. This includes a wide range of reactions in different target organs like neurological, dermal or respiratory. Chronic poisoning occurs gradually after prolonged exposure to pesticides. Increasing development of cancer and reproductive abnormalities has been seen in people who have gone through a long-term exposure to pesticides [3].

A cytogenetic study conducted in a chromatid breaks and gaps in chromosomes in the peripheral blood cells. However, the potential toxicity of residues still remained a matter of controversy. Although it is believed that adipose tissue acts as a protective reservoir.

Dichloro Diphenyl Trichloroethane (DDT) is one of the most pervasive and the persistent organic pollutant agro-chemicals with widespread negative impacts on biodiversity and human health throughout the world. It is taken up from the soil by plants, kills most invertebrates, particularly insects, and accumulates in the fatty tissues of animals, including humans, and leads to disruptions of normal breeding, particularly in animals that are high up the food chain such as birds of prey, and in mammals that are hunters. DDT is found almost everywhere in the world, even far from where it has been used as an insecticide [4].

International community through the United Nations Environment Program has developed for controlling, or rather banning the use of DDT except under special circumstances, is known as the Stockholm Convention on Persistante Organic Polutantes (POPs). All developed countries have now completely banned the use of DDT.

Ethiopia has also developed a National Implementation Plan to eliminate or minimize the use of these chemicals. However, it is still allowed to use DDT for controlling the malaria carrying a mosquito by spraying of houses once or twice a year. These spraying campaigns are not ad hoc. They are planned to give maximum protection to people living in areas where there are regular outbreaks of malaria [5].

As a result, pesticide residues in food items have been a concern to the environment and consumer groups of their wide spread use. Most pesticides especially, the organo-chlorines are very resistant to microbial degradation. They can therefore accumulate in human body fats and the environment posing problems to human health. Recent study done in Jimma zone shows some banned pesticides (DDT and Endosulfan) were detected in the pepercorn samples.

Other study done in Gurage zone showed that farmers who cultivate the Catha edulis plant in the indicated areas of Gurage zone had been using the DDT and spraying it on their Catha edulis plant to control different pests and the study revealed that the average concentrations of the first DDT metabolite, 4, 4'-DDD and the second DDT metabolite, 4, 4' of Catha edulis samples obtained from selected woredas of Gurage zone were higher than maximum allowable residual limits.

Cereal grains are the most important food grains because they are the chief source of food for the majority of the world's population. They provide about 60% of the calories and 50% of the proteins to the human race. In Ethiopia, farmers have been widely used pesticides to achieve production efficiency in cereal crops. To date, there is no study has been carried out to ascertain safety of cereal crops from both organo-chlorine and organo-phosphate pesticide residues. Therefore, the main objective of this study was to determine organo-chlorine and organo-phophate pesticide residues in selected cereal crops in Bench Maji zone, Ethiopia [6].

Materials and Methods

Description of the study area

The study was conducted in Bench Maji Zone. It is one of the 16 zones in South Nation Nationality regional. The zone has one University Teaching Hospital, 40 health centers and 182 health posts. Geographically, Bench-Maji Zone is located between 5°33' to 7°21' North latitude and 34.88° to 36°14' East longitude of the equator. The zone comprises of altitudes ranging from 1200 to 1959 meters above sea level. Besides, the mean annual temperature of the zone ranges between 15-27°C and the mean annual rainfall ranges 1500-1800 mm. According to the land utilization data of the zone, 11,383 hectares land is used for rice cultivation, 2,060 hectares for wheat production and 52,410 hectares for corn production, 3,014.75 hectares of land is for common millet production and 18,140 hectares for sorghum cultivation.

Study design and period

Experimental study design was employed to determine the types and concentration of organo-chlorine and organophosphate residues from selected cereal crops major cereal crops cultivating. The study was conducted from first.

Sample collection

The samples were collected from zone purposively selected three sample sites namely, where cereal crops are cultivated within the zone at large. Cereal crops were bought from the local farms of each. From each three sites were selected. From each site 10 samples were taken randomly and homogenized to represent the bulk sample. The bulk sample of each site was placed in net polyethylene sheets until sample preparation and analysis were done. The data handling and measurements were made as per Food and Agriculture Organization and WHO Procedural Manuals. Moreover, sample labels were properly completed [7].

Sample preparation and analysis

Sample extraction for determination of pesticides: Extraction was started with 5 g of cereal crop sample. After oven drying and grounding by mortar, 1 g of the powder was mixed with 5 ml of NaOH, sonicated for 3 minutes, and then left for 30 minutes at room temperature. Three extraction cycles were performed on the original sample with 4 ml two minutes centrifugation at 2000 g. The three extracts were combined and dehydrated with 1 g of sodium sulfate, filtered, and then reduced to 5 ml at room temperature [8].

QuEChERS method protocols: A portion of the sample was extracted using a QuEChERS Extraction Salt and Acetonitrile and centrifuged. The supernatant was treated using QuEChERS Dispersive Kit to remove interferences in the matrix and centrifuged. 0.5 mL of the supernatant was taken and 1 µl of the sample was injected to the GC-MS system. The sample chromatogram was evaluated against a calibration curve obtained from a 7 point calibration made using pure analytical standards for quantization purposes [9].

Data analysis

The pesticides residue data was analyzed statistically using Origin pro version 8.0 computer software packages. Analysis of variance (ANOVA) was used to assess the significance difference between the mean values Organo-chlorine and Organo-Phosphate pesticide residues in sample cereal crops. Possibilities less than 0.05 ($p<0.05$) was considered statistically significant and the analyzed data was presented by using. All the mean values of organo-chlorine and organophosphate pesticide residues were compared with maximum pesticides residual limits in food samples prescribed by Codex alimentary and European Union standards.

Results

A total of four cereal crops (corn, rice, common millet and sorghum) were investigated for the presence of seventeen pesticide residues. The investigated pesticide compounds were p,p'-DDT, p,p'-DDE, Chlordane, Hexachlor benzene, β -Lindane, Lindane, α -Lindane, Aldrin, hexachlorepoxide, α -Endosulfan, β -Endosulfan, Endosulfan Sulfate, Methoxychlor, Heptachlor, Dimethoate, Chlorpyrifos and Profenofos [10].

As presented in Table 1, the percentage recoveries of the pesticides standard were found to be acceptable ranging from 71.2% for β -endosulfan to 112.3% for lindane, which indicates that the reproducibility of the method was satisfactory. The limits of detection pesticides standard were ranged between 0.008-0.902 $mgkg^{-1}$ and limits of quantification varied from 0.027 to 3.022 $mgkg^{-1}$ (Table 1).

Validation result

Table 1. *Percentage recoveries and validation information of pesticides standards used for this study.*

Pesticides standard	Percentage Recovery(%)	Limits of Detection ($mgkg^{-1}$)	Limits of Quantification ($mgkg^{-1}$)
p,p'-DDE	90.5	0.01	0.033
p,p'-DDT	85.7	0.016	0.054
Chlordane	101.6	0.108	0.362
Endosulfan salfate	72.4	0.008	0.027
α -endosulfan	98.1	0.078	0.261
Hexachlor benzene	81.3	0.241	0.807
β -Lindane	73.9	0.512	2.705
Lindane	112.3	0.016	0.054
α -Lindane	82.3	0.193	0.647
Aldrin	89.4	0.02	0.067
Hexachlorepoxide	78.8	0.902	3.022
Methoxychlor	91.2	0.038	0.127
Heptachlor	81.4	0.193	0.647
Dimethoate	94.8	0.018	0.06
Chlorpyrifos	88	0.31	1.039
Profenofos	71.5	0.032	0.107
β -endosulfan	71.2	0.205	0.687

Calibration curves have been produced for quantification. Linearity has been observed all along the area of concentration studied depending on the target pesticide chemicals. These ranges of concentrations were selected in function of the sensitivity of the gas chromatography towards each pesticide from the correlation coefficient (r^2) of the linear regression. The calibration curves were obtained by injecting eight different concentrations of the pesticide standards in a range of 4-300 ng/ml. The regression coefficient (r^2) was >0.997 for all the pesticides the under study. Calibration curves of the studied analysts show satisfactory linearity over selected concentration range with regression correlation coefficients (r^2) ranging from 0.997 for p,p'- DDE, to 0.999 for Endosulfan sulfate.

Analysis of pesticide residues

Four organo-chlorine pesticide residues namely, p,p'-DDT and its metabolite (p,p'-DDE), Endosulfan salfate, and Aldrin and one organophosphate pesticide residue were detected from food samples collected from the sample sites. p,p'-DDT and p,p'-DDE were the most frequently detected contaminants in all food items obtained from Gurafarda site as shown in Table 2. Pesticide residues varied from 0.011 ± 0.003 to 0.018 ± 0.005 $mgkg^{-1}$ in corn, 0.035 ± 0.013 to 0.076 ± 0.025 $mgkg^{-1}$ in rice, 0.037 ± 0.005 to 0.068 ± 0.042 $mgkg^{-1}$ in sorghum and common millet were 0.039 ± 0.030 to 0.087 ± 0.006 $mgkg^{-1}$. Highest concentration of Endosulfan sulfate (0.076 $mgkg^{-1}$) was detected in rice, followed by Dimethoate (0.068 $mgkg^{-1}$) and p,p'-DDE (0.087 $mgkg^{-1}$) in sorghum and common millet, respectively (Table 2).

Table 2. *Concentration ($mgkg^{-1}$) of pesticide residues in assessed food items from Gurafarda site.*

Pesticide residues	Food items			
	Corn	Rice	Sorghum	Common millet
p,p'-DDT	0.018 ± 0.005	0.046 ± 0.020	0.048 ± 0.007	0.062 ± 0.027
p,p'-DDE	0.011 ± 0.003	0.035 ± 0.013	0.054 ± 0.009	0.087 ± 0.006
Endosulfan salfate	ND	0.076 ± 0.025	0.047 ± 0.013	0.039 ± 0.030
Aldrin	ND	0.06 ± 0.033	0.037 ± 0.005	0.042 ± 0.008
Dimethoate	ND	0.065 ± 0.023	0.068 ± 0.042	0.080 ± 0.018

Table 3 illustrated that, the crop samples analyzed in North-Bench site, Dimethoate was less frequently detected in corn. Highest concentration of p,p'-DDT (0.133 $mgkg^{-1}$) was detected in common millet, which indicates the recent use of the pesticide DDT in the study area.

Pesticide residues varied from 0.045 to 0.066 $mgkg^{-1}$ in corn, 0.040 to 0.077 $mgkg^{-1}$ in rice, 0.031-0.082 $mgkg^{-1}$ in sorghum and 0.018-0.133 $mgkg^{-1}$ was detected in common millet. Highest concentration of p,p'-DDT (0.133 $mgkg^{-1}$) was detected in common millet, followed by Aldrin (0.082 $mgkg^{-1}$) and Dimethoate (0.077 $mgkg^{-1}$) in Sorghum and common millet, respectively (Table 3).

Table 3. *Concentration ($mgkg^{-1}$) of pesticide residues in assessed food items from North-Bench site.*

Pesticide residues	Food items			
	Corn	Rice	Sorghum	Common millet
p,p'-DDT	0.064 ± 0.023	0.059 ± 0.26	0.031 ± 0.011	0.133 ± 0.069
p,p'-DDE	0.066 ± 0.039	0.058 ± 0.027	0.044 ± 0	0.057 ± 0.007
Endosulfan salfate	0.045 ± 0.003	0.061 ± 0.034	0.049 ± 0.018	0.018 ± 0.002
Aldrin	0.065 ± 0.028	0.040 ± 0.02	0.082 ± 0.007	0.027 ± 0.002
Dimethoate	ND	0.077 ± 0.028	0.04 ± 0.013	0.039 ± 0.006

As illustrated in Table 4, Dimethoate was detected in about 50% samples of the food items obtained from South- Bench site. It was only detected in rice (0.060 $mgkg^{-1}$) and sorghum

(0.051 mgkg^{-1}). The average residues concentration were varied from 0.056 to 0.059 mgkg^{-1} in corn, 0.035 to 0.057 mgkg^{-1} in rice, 0.051 to 0.130 mgkg^{-1} in sorghum and 0.038 to 0.074 mgkg^{-1} in common millet. Highest concentration of Aldrin residue (0.130 mgkg^{-1}) was detected in sorghum (Table 4).

Table 4. *Concentration (mgkg^{-1}) of pesticide residues in assessed food items from South- Bench site.*

Pesticide residues	Food items			
	Corn	Rice	Sorghum	Common millet
p,p'-DDT	0.056 ± 0.042	0.035 ± 0.013	0.075 ± 0.022	0.038 ± 0.016
p,p'-DDE	0.059 ± 0.023	0.057 ± 0.023	0.078 ± 0.018	0.074 ± 0.022
Endosulfan sulfate	0.081 ± 0.004	0.065 ± 0.023	0.059 ± 0.036	0.044 ± 0.020
Aldrin	0.074 ± 0.025	0.069 ± 0.027	0.130 ± 0.146	0.045 ± 0.017
Dimethoate	ND	0.060 ± 0.030	0.051 ± 0.021	ND

Discussion

In the present study, four organochlorine pesticide residues namely, p,p'-DDT and its metabolite (p,p'-DDE), Endosulfan salfate, Aldrin and one organophosphate pesticide residues were detected from food samples collected from sample sites. p,p'-DDT and p,p'-DDE were the most frequently detected contaminants in all food items. The chlorinated pesticides such as DDT and endusulfan were mostly used for malaria control in Ethiopia. This might be due to cross contamination or from their persistent nature remain inthe nearby environment.

In this study, p,p'-DDT and p,p'-DDE residues were detected in all food samples obtained from the three sites. This could be due to pesticides with high volatility can be absorbed by the foliar of non-target crops through spray drift and can also be taken up by crop roots under dry soil conditions. One other possible way by which the contamination could arise is through contaminated surfaces due to storage and distribution practices. The result of the present study is consistent with a study done in Jimma zone which reported that the concentration of DDT in coffee pulp is significantly different (p-value<0.01) from other food items except for red pepper. Although DDT is officially banned for agricultural application in Ethiopia, 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. In addition, it might be due to persistent nature of organo-chlorine pesticide residue prevail in the nearby soil and water compartment.

The only organophosphate was detectected in corn obtained from the sample sites. This is due to the fact that organophosphate compounds have the advantage of being more rapidly degraded in the environment than organo-chlorine compounds. Generally, lower concentrations Dimethoate residue was less frequently detected in all food items obtained from the three sites was above the MRL of cereals established by European Union.

The commonly consumed food items zone shows lower concentrations of p,p-DDT pesticide residues except the p,p'-DDE residue was higher concentration reported from Jimma zone, Ethiopia. This variation could be attributed to the fact that Jimma zone is a high potential area in agriculture and there is a possibility of organo-chlorine pesticide use in the past. Another reason could be leaching and surface run-off pesticides. Additionally, higher residues may result from historical use and previous environmental contamination, particularly from those compounds demonstrating environmental persistence and accumulation of obsolete pesticides nearby the study area.

The comparisons of residue concentration in food samples obtained that detected in rice, sorghum and common millet were above the MRLs set by Codex Alimentarius regulations and European Union, while the Dimethoate residue detected in rice, sorghum and common millet were contained above the MRLs set by European Union (EU). In addition, p,p'-DDT in common millet and Endosulfan sulfate in rice exceeded corresponding MRLs set by European Union.

The average concentration of p,p'-DDT and p,p'-DDE residues detected in all food samples obtained from North-Bench site were above the MRLs established by European Union and p,p'-DDT and Endosulfan sulfate residue detected in common millet and rice were also above the MRLs established by Codex Alimentarius standards and European Union, respectively. Similar result reported when compared to the study done in Jimma zone, Ethiopia which showed that DDT which was expressed as the total sum of its metabolites: p,p'-DDE, p,p'-DDD, o,p'-DDT and p,p'-DDT, was above the MRL set by Codex Alimentarius in the two commonly consumed cereals corn and teff. The reason might be due to illegal use of pesticides in the production of staple crops in the study area or there may be contamination from the environment.

Conclusion

Four cereal crops were investigated for the presence of seventeen pesticide residues. Out of them, only five pesticide residues were detected. Four organochlorine pesticide residues namely, p,p'-DDT and its metabolite (p,p'-DDE), Endosulfan salfate, and Aldrin and one organophosphate pesticide residues were detected from food samples collected from the three areas. p,p'-DDT and p,p'-DDE were the most frequently detected contaminants in all food items obtained from Gurafarda and North-Bench sites. Dimethoate was detected in 50% samples of the food items obtained from South- Bench site.

Recommendations

Tighter regulation in the production of cereal crops and implementation of integrated pest management methods should be needed. Additionally, further monitoring studies must be performed to improve food safety and protect consumers' health. In Ethiopia, there is no maximum residue limits set by the concerned bodies. Therefore, establishment of the national maximum residue limits is recommended.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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