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Determination of Selected Pesticide Residues in Tomato by Using GC - MS, Collected from Kebridehar and Gode Markets, in Somali Region, Ethiopia

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Abstract
Pesticides are commonly employed by local farmers and government entities in Ethiopia for pest control. The goal of this study was to determine the residue of organochlorine and organophosphorus pesticides in tomato samples taken from the Somali region of Ethiopia's Kebridehar and Gode markets. Ten tomato samples were gathered from local marketplaces in Kebridehar and Gode, Ethiopia, and examined for pesticide residues. Standard protocols were followed for sample collection and preparation. GC-MS was used to assess the concentrations of pesticide residues. The samples were extracted utilizing the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) approach, which included the use of a dispersive solid phase extraction cleanup process. AOAC procedure 2007.01 was used to clean up the sample extract.The samples were analyzed for organochlorine and organophosphorus pesticide residues such as **α**-BHC, β-BHC, γ- BHC *δ*- BHC, Endosulfan I (alpha), 4.4- DDE, Aldrin, Dieldrin, Endrin, Heptachlor, Endosulfan II (beta isomer), Endosulfan sulfate, 4,4- DDD, Endrin aldehyde, 4,4- DDT, Chloropyrifos Methyl, Diazinon, Ethion, Malathion, Profenophos. The analytes' percentage recoveries ranged between 74.15 and 98.47 percent. Pesticide concentrations in tomato samples were less than 0.01 mg/kg (which is below the limit of detection). The results showed that the samples were devoid of the pesticide residues that were tested.
Keywords: Pesticide residue, Tomato, QuEChERS, GC-MS, Organochlorine, organophosphorus
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1. INTRODUCTION

Tomatoes (Lycopersicum esculentum, Solanum lycopersicum, Lycopersicum lycopersicum) are a solanace-ae family member and one of the world's most extensively produced vegetables (Engindeniz, 2006 and Jahanmard *et al.,* 2016). This fruit veggie is normally grown in the spring and summer; however it is grown all year in greenhouses in several countries **(**Bidari *et al.,* 2011). Its production contributes significantly to small-scale farmers' revenue generating, job development, and foreign exchange profits. Tomato output is predicted to be at 177 million tonnes globally, with 17.2 million tonnes produced in Africa (Nakhungu *et al.,* 2021).Tomato is under the threat of various insect pests and diseases in the field, and pesticides are needed in different phases of cultivation to control pests and diseases that may cause yield reduction (Gambacorta *et al.,* 2005 and Jahanmard *et al.,* 2016).

During the production, storage, transport, distribute- on, and processing of food, agricultural commodities, or animal feeds, a pesticide is any substance or mixture of substances intended for preventing, destroying, attracting, repelling, or controlling any pest, including unwanted species of plants or animals, or which may be administered to animals for the control of ectoparasites (Ali *et al.,* 2020). Many pesticides are used to battle insect pests and illnesses of this crop and to increase output, which may leave residues on the crops. These residues, if present in large amounts, can pose a health risk to consumers and induce chronic disorders (Jahanmard *et al.,* 2016). Herbicides and insecticides are used in Ethiopia to boost agricultural yield and support public health initiatives. In rural sections of the nation, organo chlorine pesticides (OCPs) and organophosphorus pesticides (OPPs) are among the most often used pesticides. On a worldwide scale, OCPs and the breakdown products of some OPPs are classified as persistent pollutants (Debelo *et al.,* 2020). Organophosphorus pesticides have been widely utilized for a variety of vegetable and fruit crops, owing to their low environmental persistence and great efficacy in suppressing infesting pests when compared to other forms of pesticides such as organochlorine chemicals (Sharma *et al.,* 2010). Gas chromatography (GC), gas chromatography- mass spectrometry (GC- MS) (Chandra *et al.,* 2012), gas chromatogram phy ion trap mass spectrometry (GC- ITMS) (Tao *et al.,* 2009), and gas chromatography–tandem mass spectrometry (GC–MS/MS) are just a few of the standard methods available for detecting pesticide compounds in various matrices (Vidal *et al.* 2002 and Naik *et al.,* 2021). Other traditional quantification methods include highperformance liquid chromatography (HPLC) (Paranthaman *et al.,* 2012), liquid chromatography- tandem mass spectrometry (LC- MS/MS) (Hernandez *et al*., 2006 and Naik *et al.,* 2021), and low pressure gas chromatography- mass spectroscophy (LP- GC/MS) (Walorczyk *et al.,* 2006).

For many pesticides in food matrices, employing GC- MS to detect pesticide residues gives excellent identification and quantification (Jahanmard *et al.,* 2016). Various sample preparation approaches have been created in recent decades for quantification of pesticide residues in food matrices, with QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) being the most widely utilized (Salamzadeh *et al*., 2018 and Jahanmard *et al.,* 2016).The QuEChERS method is created by Anastassiades and his friends in 2003 (Anastassiades *et al.,* 2003). Because of the multistage processes and additional clean-up phases, traditional extraction techniques frequently need a high sample size. As a result, traditional procedures are time-consuming, labor-intensive, and costly, and they can generate significant waste in the environment. As a result, analytical chemists increasingly choose to employ the QuEChERS technique with a dispersive solid-phase extraction (d-SPE) clean-up to analyze a wide range of residues in food matrices (Wilkowska and Biziuk, 2011 and Mekonen *et al.,* 2014). As a result, the QuEChERS approach offers the following benefits: high sample throughput, minimal solvent and glassware consumption (no chlorinated solvents), reduced labor and bench space, lower reagent prices, ruggedness, and limited worker exposure (Anastassiades *et al.,* 2003 and Lehotay *et al.,* 2005). Pesticide residues have been found in several nations, including Ethiopia, in fruits, vegetables, and water (Loha *et al.,* 2020). Pesticides have been frequently utilized by Ethiopian farmers to increase the efficiency of cereal crop production. To far, no study has been conducted in Kebridehar and Gode Ethiopia to determine the safety of cereal crops from both organo chlorine and organophosphate pesticide residues. As a result, the goal of this study was to find pesticide residues in tomato samples obtained from Kebridehar and Gode.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

With a surface area of 350,000 kilometers square, Ethiopia's Somali regional state is the country's second biggest region, after Oromia. It has a border with the countries of Somalia, Djabouti, and Kenya. In the west, the Somali area shared a boundary with the Afar and Oromia regions. Korahay is one of the 93 districts and 11 zonal administrations of the Somali region. Kebridehar is the capital (town) of Ethiopia's Somali region, which is located in the Korahe zone of the Somali region. It has a latitude and longitude of 6°44'N 44°16'E with an elevation of 493 meters above sea level (Abdulahi *et al.,* 2020). Gode is a city in Ethiopia's Somali region, in the Shebelle zone, with a latitude of 5°57'N and a longitude of 43° 27'E. Gode is a city in Ethiopia's Somali region, in the Shebelle zone, with a latitude of 5°57'N and a longitude of 43° 27'E.

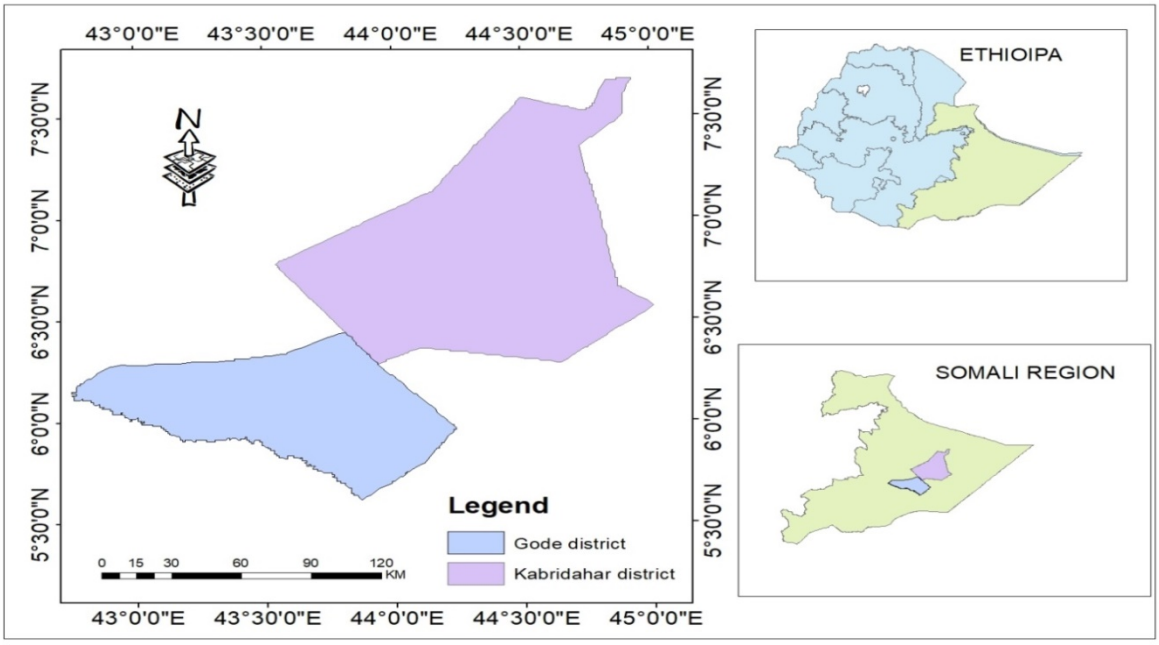

Figure 1: Location map of the study area (Source: CSA 2007, Shape file)

2.2. Study Design and Period

Organochlorine and organophosphorus pesticide residues were determined from various tomato samples taken from Kebridehar and Gode markets using a cross-sectional laboratory research approach.
The research took place from May 1st to July 31st, 2021.

2.2.1. Sample Collection

The samples were taken from two well-chosen sampling sites, namely the Kebridehar and Gode markets, where

considerable quantities of tomatoes are sold. Tomatoes were purchased from the market's local vendors. During sample collection, market vendors were asked about the origins of the tomatoes and how many farmers they purchased them from. The tomatoes were purchased from at least three farms, according to the majority of the shops. The sample was done in compliance with the European Commission (EC) directive 2002/63/EC's general principles and procedures. 10 samples (one kg each) were randomly selected from each site (5 from Kebridehar and 5 from Gode) markets and placed in polythene bags in ice-filled boxes to minimize contamination and degradation, tagged, and transferred to the laboratory where they were maintained at -20°C until analysis.

2.3. Apparatus

Pesticide residue analysis was performed using an analytical balance, refrigerator, House Hold Mill (Stainless Steel Knives), vortex mixer, centrifuge tube, gas chromatograph, GC (model 7890B manufactured by Agilent technology), and triple quadruple mass spectrometer, MS (model 7000C manufactured by Agilent technology).

2.4. Chemicals and Reagents

All organic solvents used for extraction were HPLC grade and purchased from different suppliers and importers found in Ethiopia. The pesticide standards were obtained from PIPARK Scientific Limited, Northampton, UK and all of them have analytical standard grade.

Pesticide reference standards with their purity includes; 4,4- DDT(99%), 4.4- DDE(99.9%), 4,4- DDD(98%), α- BHC(98%), β- BHC(99.5), γ- BHC(99.9%), δ- BHC(99.5%), Aldrin(97%), Heptach-lor (99.5%), Endosulfan I(alpha)(98.8%), Endosulfan II (beta)(99.5%), Endosulfansulfate(98.8%), Dieldrin(95%), Endrin aldehyde (95%), Endrin(98%), Diazinon(90%), Chloropyrifos methyl(99.5%), Ethion(98.5%), Malathion(99.5%) Profenofos (97.9%).All the solvents and reagents like acetonitrile (99.9% for HPLC), n- hexane (99%), acetone (99%),magnesium sulfate (99% laboratory reagent), sodium acetate (99%), PSA (100%), and methanol (99.9%) were obtained from Sigma Aldrich. Co., Germany.

2.5. Sample Extraction and Clean Up Procedure

The samples were extracted and cleaned using a modified QuEChERS procedure combined with the d-SPE clean-up approach. The processes were based on the official method 2007.01 of the Association of Analytical Communities (AOAC) (Lehotay, 2007). The following is the spiking and extraction procedure: Pesticide residues were extracted from fresh tomatoes (1 kg) from each sample. The sample was minced and homogenized with a stainless steel knife, and 15g of the homogenized sample was weighed into a 50 ml centrifuge tube, 15 ml of acetonitrile containing 1% acetic acid was added, and the mixture was vortex mixed for 2 minutes to ensure that the solvent and the sample were in contact, and 0.15 g anhydrous $MgSO_4$ and 2.5 g sodium acetate monohydrate were added and the sample was mixed for 2 minutes in a vortex mixer before being centrifuged for 10 minutes at 4000 rpm. The samples were centrifuged at 4000 rpm for 10 minutes to remove coextractives from the matrix, and 6 ml of the supernatant was transferred to a 15 ml centrifuge tube containing 0.15 g $MgSO_4$ and 0.05 g PSA (primary secondary amine). The extract was agitated for 2 minutes with a vortex mixer, and then centrifuged at 4000 rpm for 10 minutes before being poured into an autosampler vial for GC-MS analysis.

2.6. Identification of Pesticides by comparing the retention time of the chemical to that of a technical grade reference standard, the compound was identified.

2.7.Analytical Method Validations

2.7.1. Linearity of the Calibration Curves

Analytical solutions of a combination of pesticides made in pure solvent and created in a matrix extract in the concentration range were used to create the calibration curves. A correlation value of more than 0.999 is typically regarded as satisfactory (Zelelew *et al,* 2018). 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 and the values were between 0.995 and 0.999. The calibration curves for each analyte were linear and obtained by injecting five differe-nt concentrations in a range of 10-50 ng/ml.

2.7.2. Recovery studies

The QuEChERS approach for determining pesticide residues in various foods has been shown to provide a greater recovery than traditional liquid–liquid extraction procedures (Mekonen *et al.,* 2014). The modified QuEChERS technique is based on the official method 2007.01 of the Association of Analytical Communities (Lehotay, 2007 and Mekonen *et al.,* 2014). The percentage recovery was used in this investigation to see how effective the proposed strategy was at extracting data. Blank tomato samples were spiked, and the results in Table 1 revealed that analyte percentage recoveries ranged from 74.15 to 98.47 percent. The concentration of each analyte was recovered from tomato samples within the permissible recovery range of 70 to 120 percent using a matrix matched calibration curve (Hamilton *et al*., 2003).

Table 1: The Percentage recoveries and validation of pesticide standards

S/No	Pesticide standard	Limits of detection ($mgkg^{-1}$)	Limits of quantification ($mgkg^{-1}$)	Recovery (%)
1	α-BHC	0.195	0.657	79.03
2	β-BHC	0.492	2.603	78.20
4	γ-BHC	0.013	0.052	82.66
4	δ- BHC	0.018	0.059	86.83
5	Heptachlor	0.189	0.653	82.26
6	Aldrin	0.022	0.069	81.03
7	Endosulfan sulfate	0.008	0.029	90.70
8	Endosulfan I (alpha)	0.072	0.259	85.67
9	4.4-DDE	0.013	0.040	89.27
10	Dieldrin	0.023	0.059	93.24
11	Endrin	0.020	0.063	82.57
12	Endosulfan II(beta)	0.195	0.637	90.47
13	4,4-DDD	0.015	0.038	95.07
14	Endrin aldehyde	0.019	0.032	85.22
15	4,4-DDT	0.018	0.064	98.47
16	Diazinon	0.018	0.060	83.79
17	Malathion	0.013	0.052	74.15
18	Chlorpyrifos-methyl	0.310	1.039	78.93
19	Ethion	0.020	0.132	81.96
20	Profenofos	0.032	0.107	95.65

BHC= Benzene hexachloride, DDE = Dichloro- diphenyldichlorethylene, DDD = Dichloro- diphenyldichlorethane, DDT =Dichloro- diphenyltrichloroethane.

Table 2: Linearity and correlation coefficient (R^2)

S/N	Pesticide residues	Equation of regression	Correlation coefficient (r^2)
1	α-BHC	Y=384.76x-1040.4	0.996
2	β-BHC	Y=1021.3x-7809.1	0.995
3	γ-BHC	Y=14759x-33202	0.997
4	δ-BHC	Y=7352.4x-12389	0.998
5	Heptachlor	Y=11700x-37009	0.997
6	Aldrin	Y= 6663.8x-28216	0.996
7	Endosulfan sulfate	Y=15824-39295	0.998
8	Endosulfan I(alpha)	Y=4860.3x-7602.4	0.998
9	4,4-DDE	Y=4270.2x-5340	0.999
10	Dieldrin	Y=8135x-12290	0.995
11	Endrin	Y=3871.4x-6257.6	0.996
12	Endosulfan II(beta)	Y=2943.6x+8416.9	0.998
13	4,4-DDD	Y=2557.7x-5125.2	0.997
14	4,4-DDT	Y=6511.6x-28684	0.996
15	Endrin aldehyde	Y=3233.8x-12405	0.998
16	Diazinon	Y=6511.6x-28684	0.996
17	Malathion	Y=5781.8-16950	0.997
18	Chlorpyrifos-methyl	Y=13594x+26090	0.997
19	Ethion	Y=3098.5x+20625	0.998
20	Profenophos	Y=13594x+26090	0.998

3. RESULT AND DISCUSSION

3.1. Pesticide Residues in Tomato Samples

According to the procedure outlined, ten (10) samples were evaluated for twenty (20) pesticide residues. It was obtained that all of the samples were free of Organochlorine pesticides such as 4,4-DDT, 4,4- DDE,4,4-DDD, α-

BHC, β BHC, δ BHC, BHC, Aldrin, Heptachlor, Endosulfan I (alpha), Endosulfan II (beta), Endosulfan sulfate, Dieldrin, Endrin, Endrin aldehyde, and organophosphorus pesticide residues such as, Diazinon, Chloropyrifos methyl, Ethion, Malathion, Profenophos were analyzed in the samples and the concentration of both pesticides residues were less than 0.01 mg/kg which is the detection limit of the instrument. Therefore the pesticides were not detected in the tomato samples (below LOD).Table 3 shows that the samples tested were not be contaminated by pesticide residues.

Table 3: Concentration and linearity of pesticide residues assessed in both site

S/No	pesticide residues	Pesticide group	Concentration (mg/kg)
1	α-BHC	Organochlorine	Not detected
2	β-BHC	Organochlorine	Not detected
3	γ-BHC	Organochlorine	Not detected
4	δ-BHC	Organochlorine	Not detected
5	Heptachlor	Organochlorine	Not detected
6	Aldrin	Organochlorine	Not detected
7	Endosulfan sulfate	Organochlorine	Not detected
8	Endosulfan I(alpha isomer)	Organochlorine	Not detected
9	4,4-DDE	Organochlorine	Not detected
10	Dieldrin	Organochlorine	Not detected
11	Endrin	Organochlorine	Not detected
12	Endosulfan II (beta isomer)	Organochlorine	Not detected
13	4,4-DDD	Organochlorine	Not detected
14	4,4-DDT	Organochlorine	Not detected
15	Endrin aldehyde	Organochlorine	Not detected
16	Diazinon	Organophosphorus	Not detected
17	Malathion	Organophosphorus	Not detected
18	Chlorpyrifos-methyl	Organophosphorus	Not detected
19	Ethion	Organophosphorus	Not detected
20	Profenophos	Organophosphorus	Not detected

3.2. Chromatographic Identification

By comparing the retention times of the target analytes in the tomato sample to the retention times in the standard solution's chromatogram, the presence of the target analytes in the tomato sample was determined. All chromatograms of the combination of pesticide standards, as shown in figure 1, contain distinct and good chromatographic peaks for organochlorine pesticides with their retention period (min).

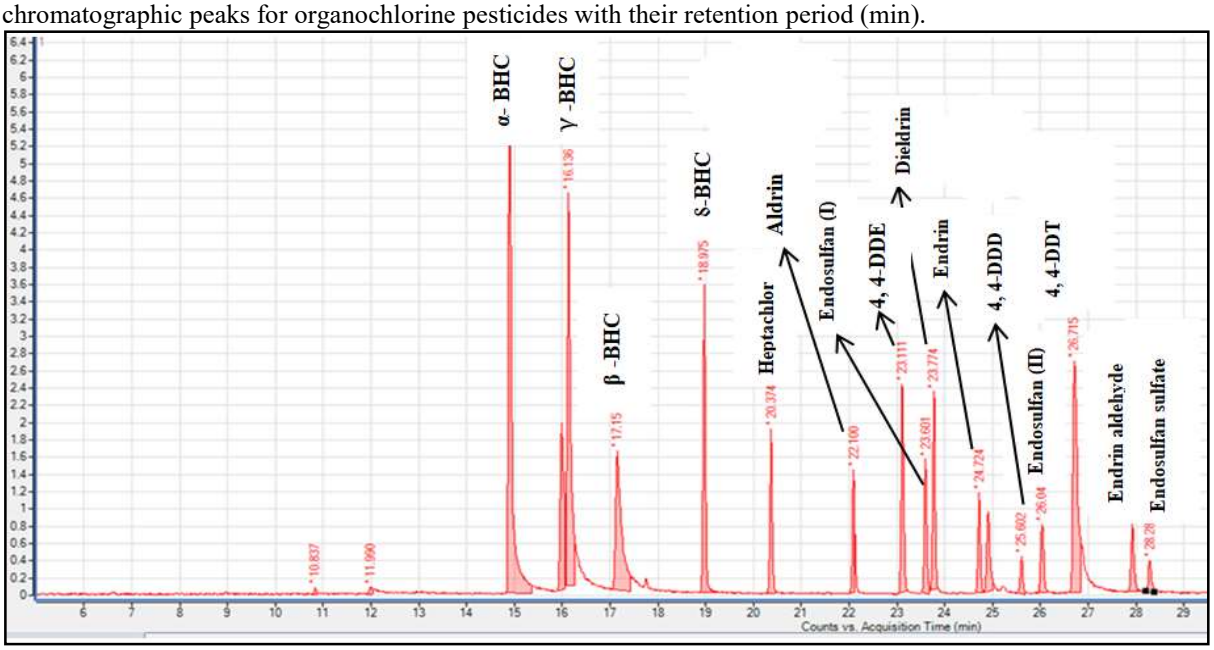

Figure 1: Chromatogram of organochlorine pesticides standards (Counts vs Acquisition time (min))

As shown in the Figure 2, below, the tomato samples collected from Kebridehar and Gode market were analyzed by GC MS. The samples were assessed for 15(fifteen) organochlorine pesticide residues, namely, α-BHC,β-BHC, γ-BHC, δ-BHC, Endosulfan I (alpha), 4.4-DDE, Dieldrin, Endrin, Endosulfan II (beta isomer),4,4-DDD, Endrin aldehyde, 4,4-DDT. The retention times of the analyses detected in the samples were not be visible

as those of standards therefore this indicates that none of these pesticides was detected in the tomato samples.

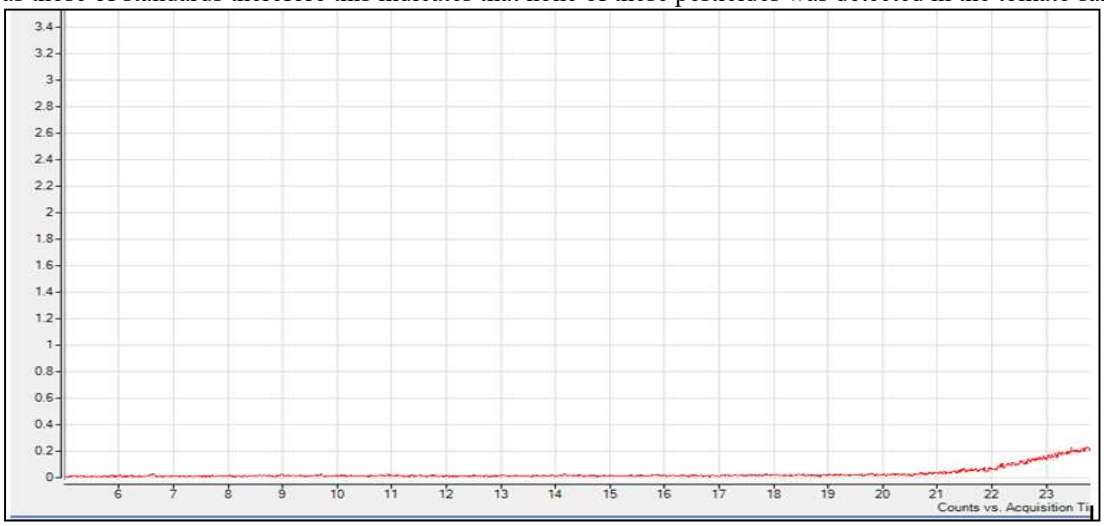

Figure 2: Chromatogram of tomato samples (Counts vs Acquisition time (min))

As shown in Figure 3 Chromatograms of mixture of organo phosphorus pesticides standards, have clear and excellent chromatographic peak with their retention time (min)

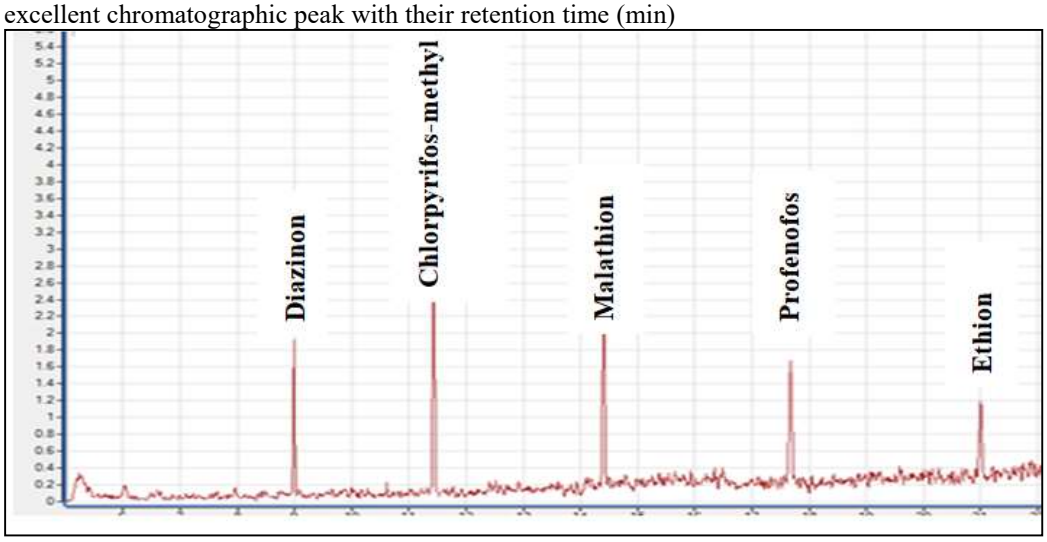

Figure 3: Chromatogram of organophosphorus pesticide standards

As shown in the Figure 4, the tomato samples were assessed for 5 organophosphorus pesticide residues, namely, Diazinon, Malathion. Chlorpyrifos-methyl, Ethion and Profenofos and none of these pesticides was detected in the analysis and the retention times of the analyses in the samples were not be visible as those of standards therefore the results confirmed that pesticide residues were absent in the samples in Kebridehar and Gode.

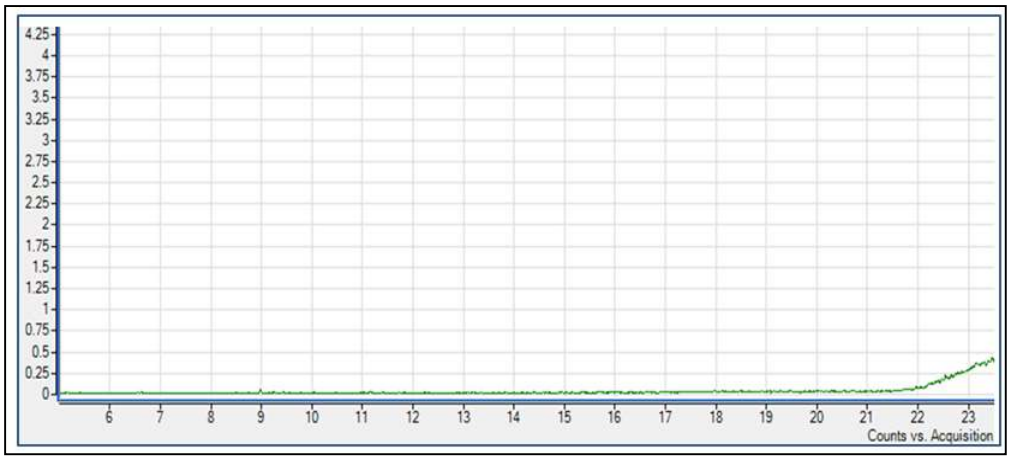

Figure 4: Chromatogram of tomato samples (Counts vs Acquisition time (min))

CONCLUSION

This study determined the levels of pesticide residues in tomato samples in Kebridehar and Gode Ethiopia. A rapid and sensitive analytical method for determination of pesticides in tomato was validated. For extraction and clean-up, the modified QuEChERS method with d-SPE was used and the procedures were based on Association of Analytical Communities official (AOAC) method 2007.01. The modified QuEChERS sample preparation and quantification by GC MS showed linearity (R^2) between 0.995 and 0.999. The percentage recovery was conducted to observe the extraction efficiency of the proposed method and it was in the range of 74.15 - 98.47%. A total of 10 tomato samples were monitored using this validated method and it was revealed that all the samples collected from the local market in Kabridahar and Gode Ethiopia are free of the analyzed pesticides residues (showed low levels of pesticide residues, below the limits of detection (LOD) or the samples were not infected by pesticide residues and are healthy for consumers.

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CONFLICT OF INTERESTS

The authors have declared there is no conflict of interests.

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