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A validated multi-residue method for the determination of 34 pesticide residues in different types of tea bags using QuEChERS method with LC-ESI-MS/MS

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Abstract

In this study a multi-residue method was used for the analysis of pesticide residues in several types of tea bags using QuEChERS method. Phase separation and pesticide partitioning were performed by the addition of the extraction kits based on the EN-QuEChERS methodology. The LC-MS/MS analysis was conducted using an Agilent HPLC 1260 along with API 6500 QTRAP mass spectrometer and electro spray ionization (ESI) interface. The chromatographic separation was achieved using an Agilent C18 reverse-phase column ZORBAX Eclipse plus 4.6 × 150 mm with 5.0 µm particle size. The ESI was operated in the positive mode. A gradient elution program was used at 500 µL/min flow rate using 10 mM ammonium formate in 1% methanol and methanol. The obtained results showed that, more than 34 pesticides have been detected in most of examined samples collected from different companies in the Egyptian market. Limit of quantitation for the studied pesticides was10µg/kg. It was found that some pesticide residues exceed Maximum Residue Level (MRL). The optimized QuEChERS was validated according to accuracy, precision, detection limit (LOD), quantification limit (LOQ) and it was successfully applied for the detection of 34 pesticide residues in tea bags samples collected from the Egyptian market.

Keywords: Pesticide residues- Tea bags- QuEChERS method- LC-ESI-MS/MS.

1. Introduction

Every day, million cups of tea are consumed as a morning drink by two-third of the world population (Kumar & Joshi *et al.*, 2016), and mostly they are in the form of tea bags because of their convenient nature, easy to dispose-off, and blending of many ingredients can be done in a small pack of tea bag that is easy to handle and easy to prepare (Bassi *et al.*). Based on the processing techniques and type of fermentation, teas, formed of *Camellia sinensis* leaves, are classified into three major categories that is, green tea (nonfermented), oolong tea (semifermented), and black tea (fully fermented) of which the most widely used is the green tea because of its various health benefits (Ho *et al.*,

2008). Alongside the ever-popular green and black varieties, tea can be made with water infusions of the roots, leaves, flowers and other component parts of a hugely diverse range of plant species such as Hibiscus, Cinnamon, Ginger, Peppermint, Anise, Talia, Caraway, and Chamomile and are synonymously referred as "Herbal tea". Herbal teas have long-since been used as therapeutic vehicles in Chinese, Indian and other indigenous medical systems. Nowadays, they are acknowledged with their numerous health benefits such as achieving a more calm and relaxed state of mind, supporting heart health, aiding with stomach and digestive problems, strengthening the immune system, providing antioxidants to the body, relieving stress, and many others (Poswal *et al.*, 2019).

The climatic conditions of the tea growing regions are conducive for many insects and mite pests, diseases and weeds that needs to be managed below the economic injury levels to avoid huge crop loss. Therefore, it's far essential to apply broad-spectrum artificial insecticides, consisting of organochlorine insecticides (OCPs), organophosphorus (Ops), artificial pyrethroids (SPs), carbamates, herbicides and neonicotinoids which might be endorsed for the duration of cultivation to guard their fields (Cajka *et al.*). Unfortunately, the chemicals in the pesticides are absorbed and stored by the plant, which in turn infuse into the brewed tea resulting in numerous health problems such as cancers of the lung, prostate, and lymphatic and hematopoietic machine (Gupta *et al*, 2007, Jaga, and Dharmani *et a.l*, 2006, Baker *et al*, 1988). Therefore, many countries have maximum levels of allowable residual pesticides in tea (MRLs).

A lot of work has been conducted in the pesticide residues analysis in food as well as medicinal plant matrices. A new rapid method for the determination of 135 pesticide residues in green and black dry tea leaves and stalks employing gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) with a triple quadruple was developed and validated (Cajka *et al*., 2012).

An efficient and sensitive method for simultaneous determination of 118 pesticide residues in teas has been established and validated. The method involved extraction with ethyl acetate–hexane, clean-up using gel permeation chromatography (GPC) and solid-phase extraction (SPE), and subsequent identification and quantification of the selected pesticides by gas chromatography–mass spectrometry (GC–MS) (Yang *et al*., 2009).

The AOAC collaborative investigation has evaluated the buffered QuEChERS method for 20 represented pesticides in vegetable and fruits (Lehotay *et al*. 2007).

However, a wide range of interfering compounds could be found in dry herbs when detecting pesticides due to high extractives content and less content of water (Lozanoa *et al*., 2012).

A modification of QuEChERS method to analyse dry herbal products was done by adding 30-min hydration step (Moreno-González *et al*., 2015). In another study 10-min hydration step was used (PérezParada *et al*., 2018).

Sonication was found better than the dispersive solid material in extraction (Capriotti *et al*., 2010). The dispersive solid phase extraction (D-SPE) was introduced as a cleanup step and helped in cell membranes disruption (Barker *et al*., 1989) like Octadecylbonded silica (C18) which was used as lipophilic sorbent material (Barker *et al*. 2007). Primary secondary amine (PSA) was used to remove interfering matrix with acid characteristics (Sampaio *et al*., 2013).

A cleanup study investigated the use of PSA, C18 and graphitized carbon black (GCB) as D-SPE, individually and together in different matrices on 25 moderately polar pesticides resulting in acceptable recovery (Qin *et al*., 2015).

Hydrophilic lipophilic balanced polymer (HLB) was used in the determination of 52 pesticides with different families as SPE cleanup step and resulted in acceptable recoveries (Hernández *et al*., 2006).

The principal aim of this study is to develop a fast and sensitive QuEChERS method with LC-ESIMS/MS and GC-MS/MS, for the identification of a wide range of pesticides.

Accordingly, we used 34 pesticides; namely: (Acetamiprid, Atrazine, Azoxystrobin, Boscalid, Carbaryl, Carbendazim, Chlorfenapyr, Chlorpyrifos, Cyfluthrin, Cypermethrin, Diazinon, Difenoconazole, Dimethomorph, Flusilazole, Imidacloprid, LambdaCyhalothrin, Malathion, Metalaxyl, Myclobutanil, Penconazole, Pendimethalin, Piperonyl butoxide,, Profenofos, Propamocarb, Propargite, Propiconazol, Pyraclostrobin, Sulfur, Tebuconazole, Tetraconazole, Thiacloprid, Thiophanatemethyl and, Trifloxystrobin). These were studied whether being detected in different types of tea bags in the Egyptian market.

2. Materials and methods

2.1. Sample Collection

A total of twenty-five samples of different types of tea bags Green Tea with Mint, Anise, Cinnamon, Hibiscus, Ginger, Talia, Tea with mint, Black tea with cardamom, Talia Guava, Peppermint, Caraway, Chamomile, and Lemon with Ginger. The brands were collected from the Egyptian market during the period from January to June 2021. Each tea sample was labeled with a unique code. The details of the number of samples' codes are summarized in Table 1.

2.2. Apparatus

The homogenization of the samples was done using the Grindomix Knife Mill GM 300 grinding machine from Retsch® (Düsseldorf, Germany). The polytetrafluoroethylene (PTFE) membrane syringe filter with 0.45 µm pore size and the 50 ml polypropylene centrifuge falcon tubes with screw caps were from Supelco® (Bellefonte, USA). The centrifuge used was the Z32 HK from Hermle® (Gosheim, Germany). The injection vials were glass and were brought along with teflon coated caps from Agilent technologies (Santa Clara®, CA, USA). The volumetric flasks (5, 10 and 20 ml), the graduated glass pipettes (5 ml), the bottle top dispenser (5-50 ml) and the micropippettes (variable 2-20 µl, 10-100 µl and 100-1000 µl) used in this study are from Hirschman® (Eberstadt, Germany). The rotary evaporator used was the Hei-VAP value provided by Heidolph® (Schwabach, Germany). The bench top pH-meter, analytical balance and precision balance were from MettlerToledo® (Greifensee, Switzerland).

2.3. Chemicals and reagents

All pesticide reference standards(Acetamiprid, Atrazine, Azoxystrobin, Boscalid, Carbaryl, Carbendazim, Chlorfenapyr, Chlorpyrifos, Cyfluthrin, Cypermethrin, Diazinon, Difenoconazole, Dimethomorph, Flusilazole, Imidacloprid, LambdaCyhalothrin, Malathion, Metalaxyl, Myclobutanil, Penconazole, Pendimethalin, Piperonyl butoxide,, Profenofos, Propamocarb, Propargite, Propiconazol, Pyraclostrobin, Sulfur, Tebuconazole, Tetraconazole, Thiacloprid, Thiophanatemethyl and, Trifloxystrobin) were obtained from Dr. Ehrenstorfer® (Germany). Acetonitrile was purchased from J.T. Baker (USA). Toluene, acetone, ethyl acetate and n-hexane were all brought from Merck (Germany). Citrate-buffer-based QuEChERS (EN-QuEChERS) extraction and primary secondary amine bonded phase silica (PSA) dispersive solid phase extraction (D-SPE) kits were supplied by Agilent (USA). Other used chemicals in this study were brought from Sigma-Aldrich (Canada). Water was deionized in the laboratory using a Millipore (USA) MilliQ water purification system.

2.4. Sample preparation using QuEChERS method

Two grams of each sample were weighed into 50-ml polypropylene centrifuge tubes. Then, 10 ml of deionized water was added to the sample followed by shaking for 1 minute using a mechanical shaker. Ten milliliters of Acetonitrile were then added, and the sample mixture was shaken vigorously for one minute. Phase separation and pesticide partitioning were performed by the addition of the extraction kits based on the EN-QuEChERS methodology [4 g magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate tribasic dihydrate (SCTD) and 0.5 g sodium citrate dibasic sesquihydrate (SCDS)]. The tube was closed, shaken for another min, and centrifuged for 5 min at 4500 RPM. Five milliliters of the supernatant were then transferred into another 15 ml capacity centrifuge tube containing 900 mg anhydrous magnesium sulfate and 150 mg PSA for D-SPE. The tube was shaken vigorously for 1 min and then centrifuged at 4500 Rpm for 2 min. The solvent exchange was performed by evaporating 2 ml of the supernatant in a 100 ml flat bottom flask at 39°C and 200 Rpm using the rotary evaporator till dryness. Reconstitution using 2 ml n-hexane: acetone (9:1 v/v), followed by ultrasonication for 30 s. Filtration through a 0.45 µm syringe filter was then done into an auto-sampler vial for LC/MS-MS analysis.

2.5. Pesticide standard preparation

Stock solutions of 1 mg/ml for each pesticide (Acetamiprid, Atrazine, Azoxystrobin, Boscalid, Carbaryl, Carbendazim, Chlorfenapyr, Chlorpyrifos, Cyfluthrin, Cypermethrin, Diazinon, Difenoconazole, Dimethomorph, Flusilazole, Imidacloprid, Lambda Cyhalothrin, Malathion, Metalaxyl, Myclobutanil, Penconazole, Pendimethalin, Piperonyl butoxide,, Profenofos, Propamocarb, Propargite, Propiconazole, Pyraclostrobin, Sulfur, Tebuconazole, Tetraconazole, Thiacloprid, Thiophanatemethyl and, Trifloxystrobin) were prepared in toluene and a 2.5 µg/ml composite standard solution of all the studied pesticides were also prepared in toluene. The calibration solutions were prepared in n-hexane: acetone (9:1) at concentration level of 0.001,0.01,0.03,0.05,0.08 and 0.1µg/ml.

Table 1: Code, Name, and Production date of the studied samples

NO	Sample	Name	Production date
1	**S1**	Green Tea with Mint	7/2020
2	**S2**	Anise	3/2021
3	**S3**	Cinnamon	8/2020
4	**S4**	Hibiscus	11/2019
5	**S5**	Ginger	6/2020
6	**S6**	Talia	10/2020
7	**S7**	Green tea with mint	4/2021
8	**S8**	Black tea with cardamom	12/2020
9	**S9**	Talia Guava	6/2020
10	**S10**	Green tea	7/2020
11	**S11**	Black tea	3/2021
12	**S12**	Black tea	4/2020
13	**S13**	Mint	9/2020
14	**S14**	Tea with mint	4/2020
15	**S15**	Cinnamon	10/2020
16	**S16**	Hibiscus	1/2020
17	**S17**	Peppermint	4/2021
18	**S18**	Caraway	9/2020
19	**S19**	Green Tea	2/2020
20	**S20**	Cinnamon	12/2020
21	**S21**	Anise	1/2021
22	**S22**	Hibiscus	6/2020
23	**S23**	Chamomile	11/2020
24	**S24**	Green Tea	10/2020
25	**S25**	Lemon with Ginger	11/2020

Table 2: LC/MS/MS conditions

Total time (min)	Flow Rate (µL/min)	A%	B%
0	500	50	50
2	500	50	50
3	500	25	75
10	500	25	75
10.5	500	10	90
11	500	3	97
17.5	500	3	97
17.6	500	50	50

Where A: 10 mM ammonium formate solution in methanol: water (1: 9 v/v); B is methanol

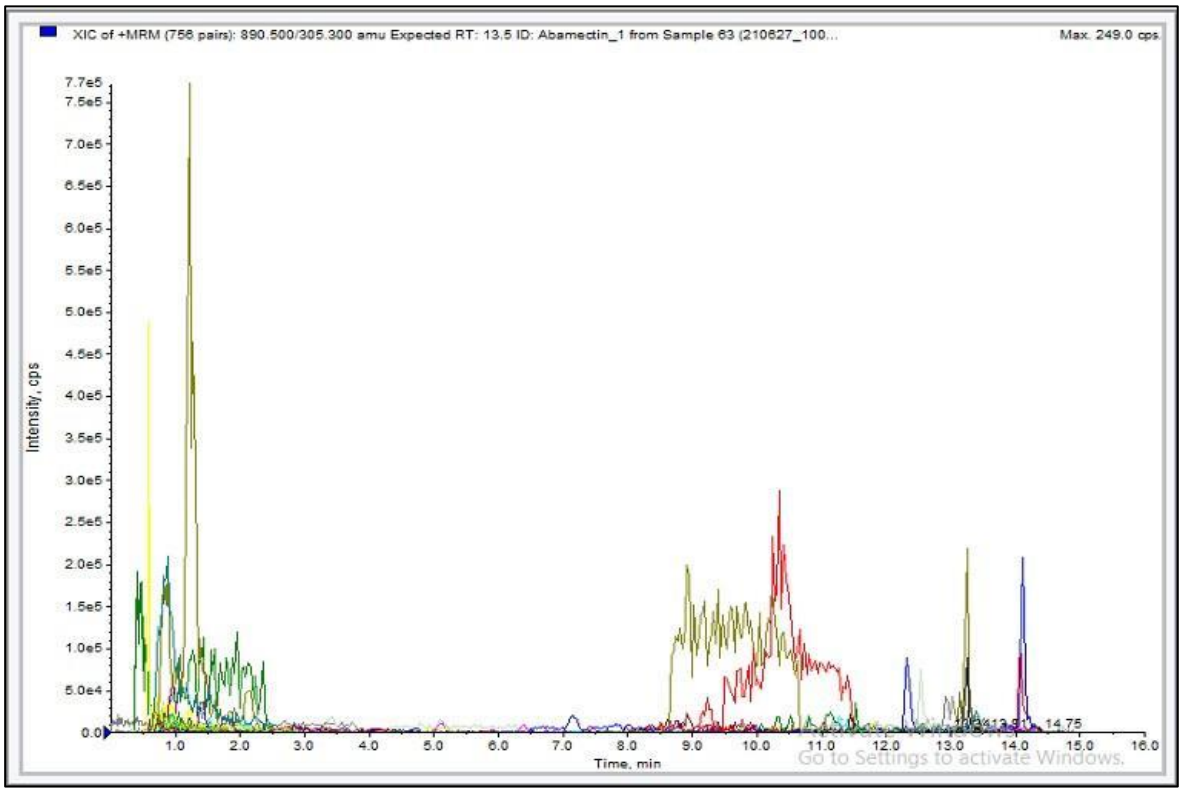

Fig. 2: LC-MS/MS Chromatogram of Hibiscus sample No 4.

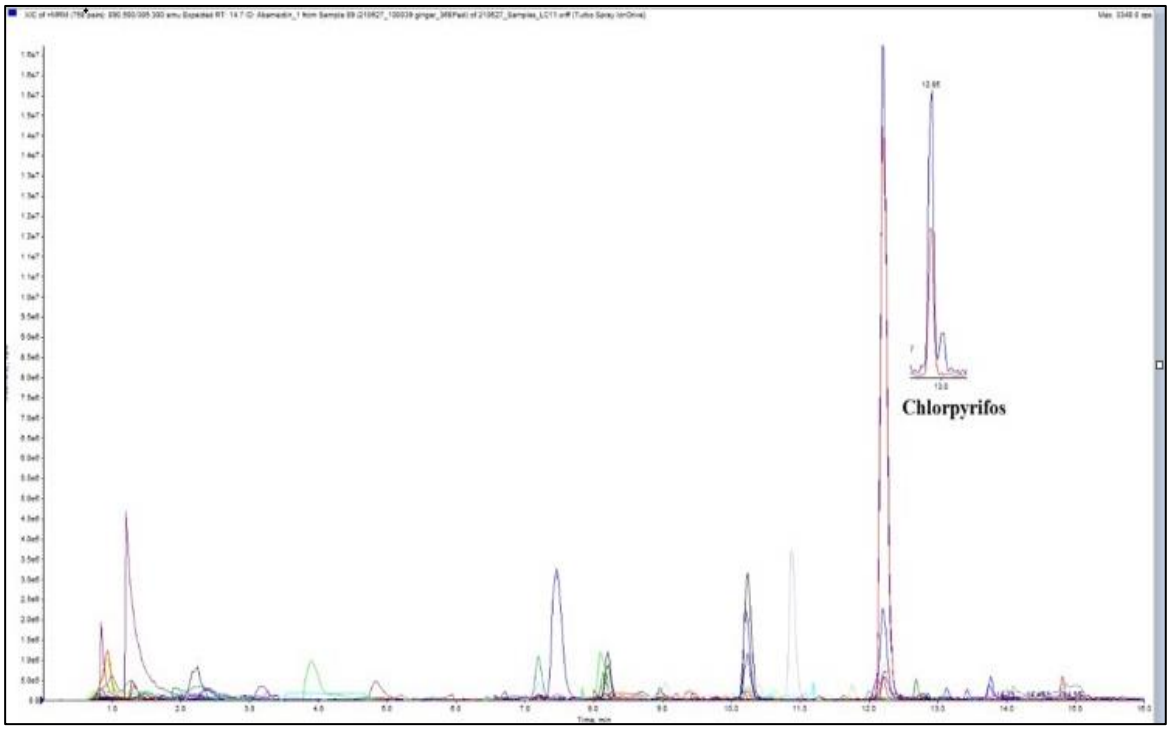

Fig. 3: LC-MS/MS Chromatogram of Ginger sample No 5.

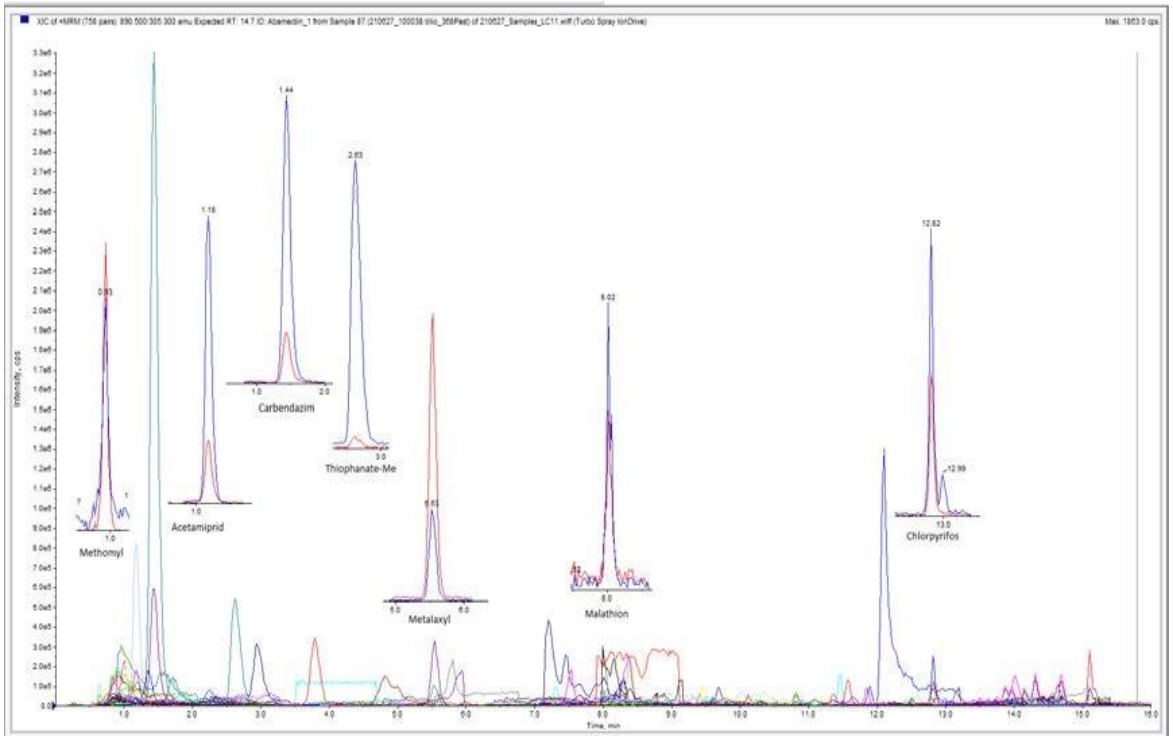

Fig. 4: LC-MS/MS Chromatogram of Tilio sample No 9

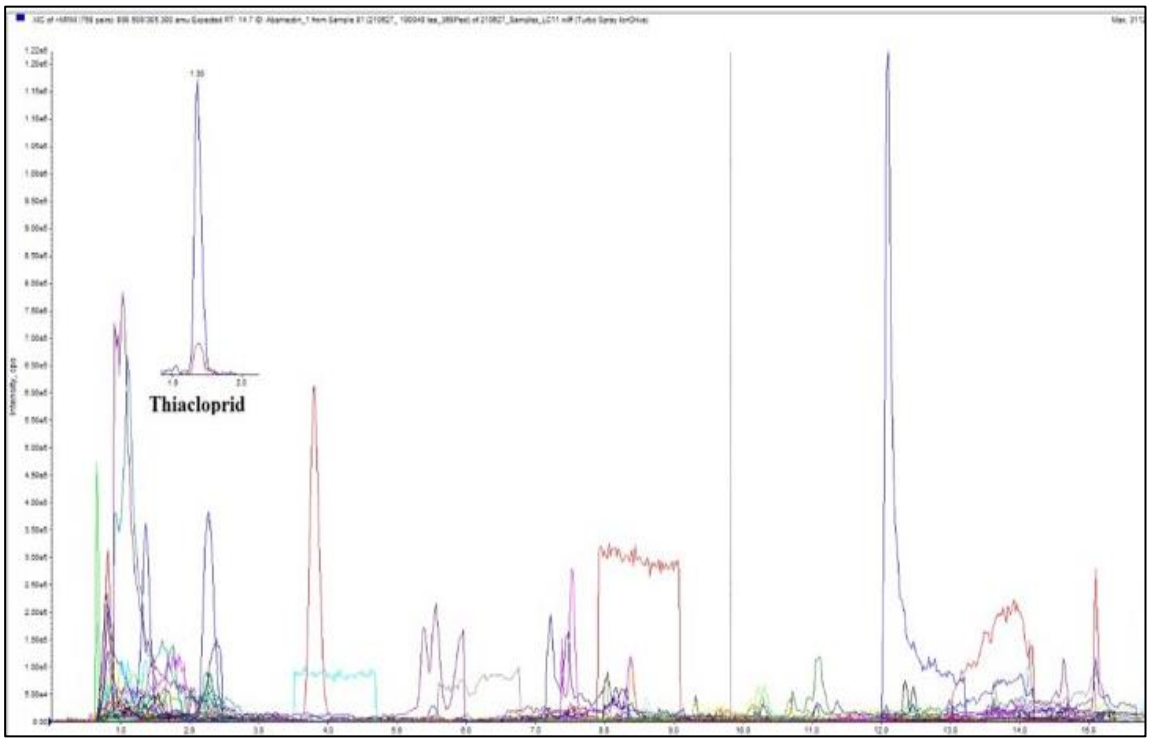

Fig. 5: LC-MS/MS chromatogram of Black tea sample No 12

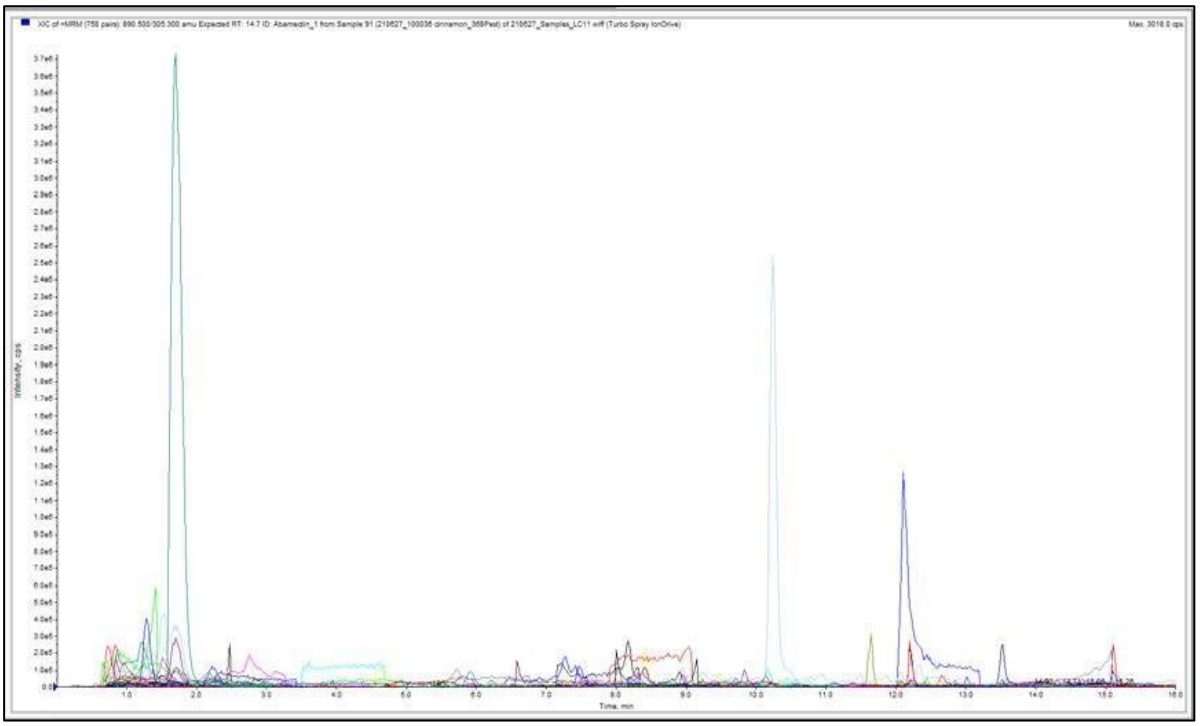

Fig. 6: LC-MS/MS Chromatogram of Cinnamon sample No 15.

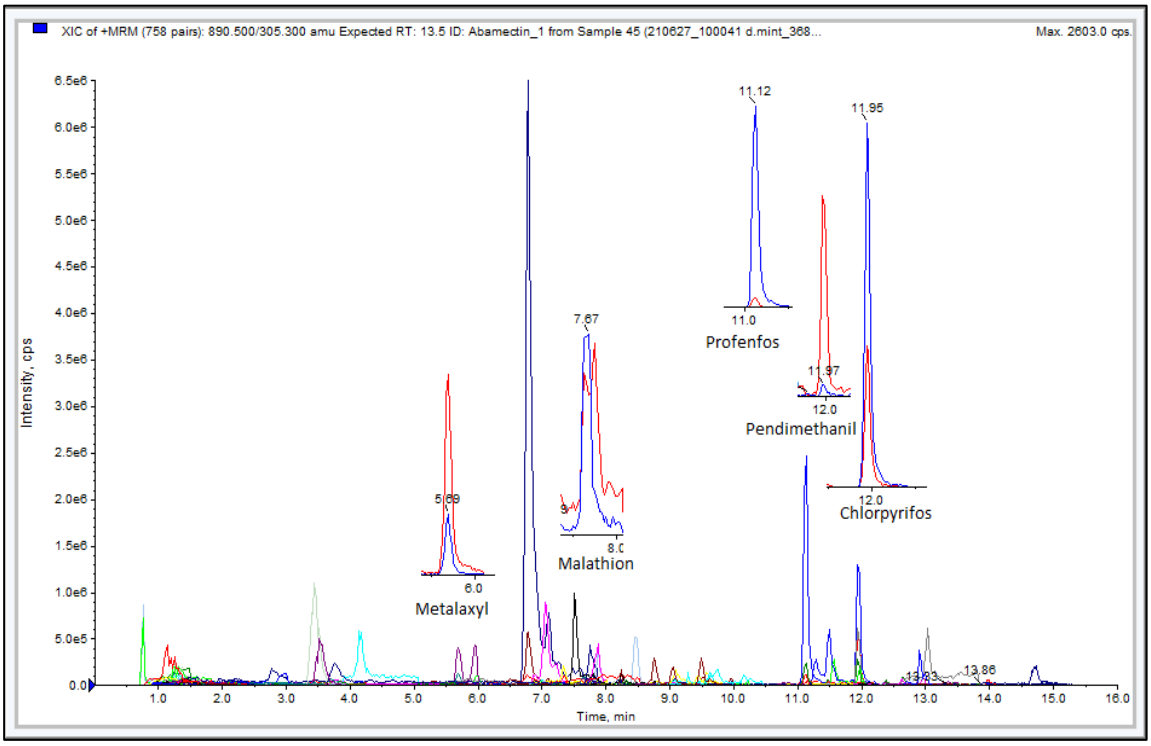

Fig. 7: LC-MS/MS Chromatogram of Dry Peppermint sample No 17

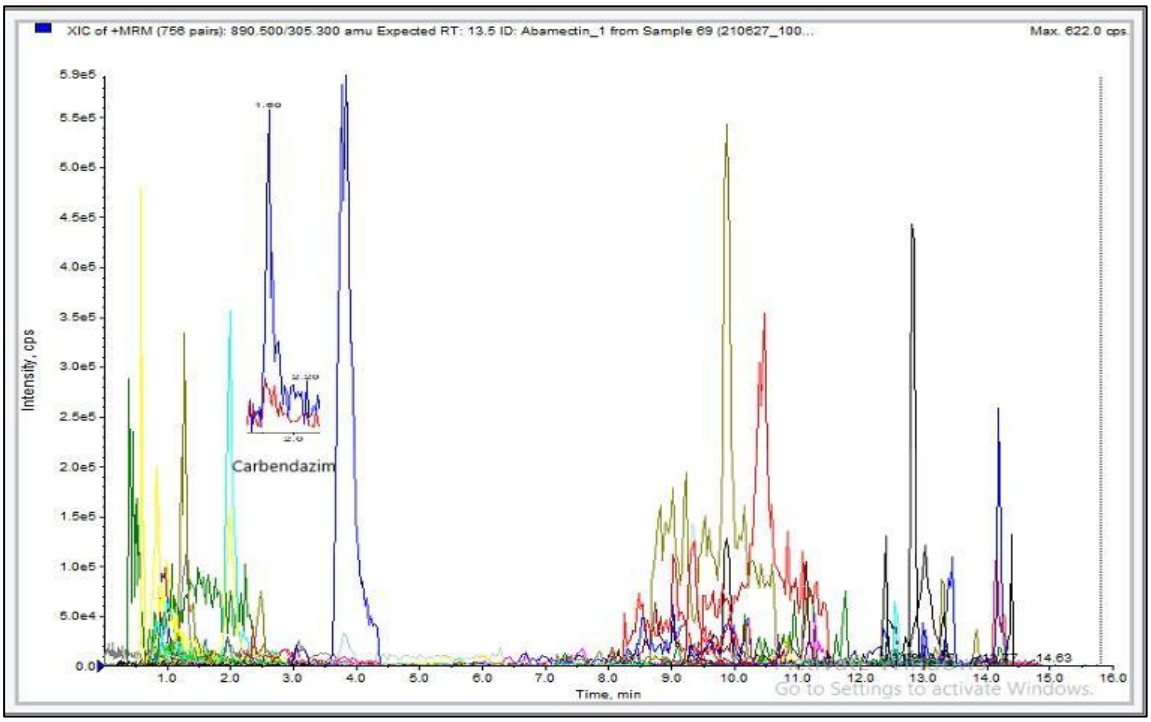

Fig. 8: LC-MS/MS Chromatogram of Caraway sample No 18.

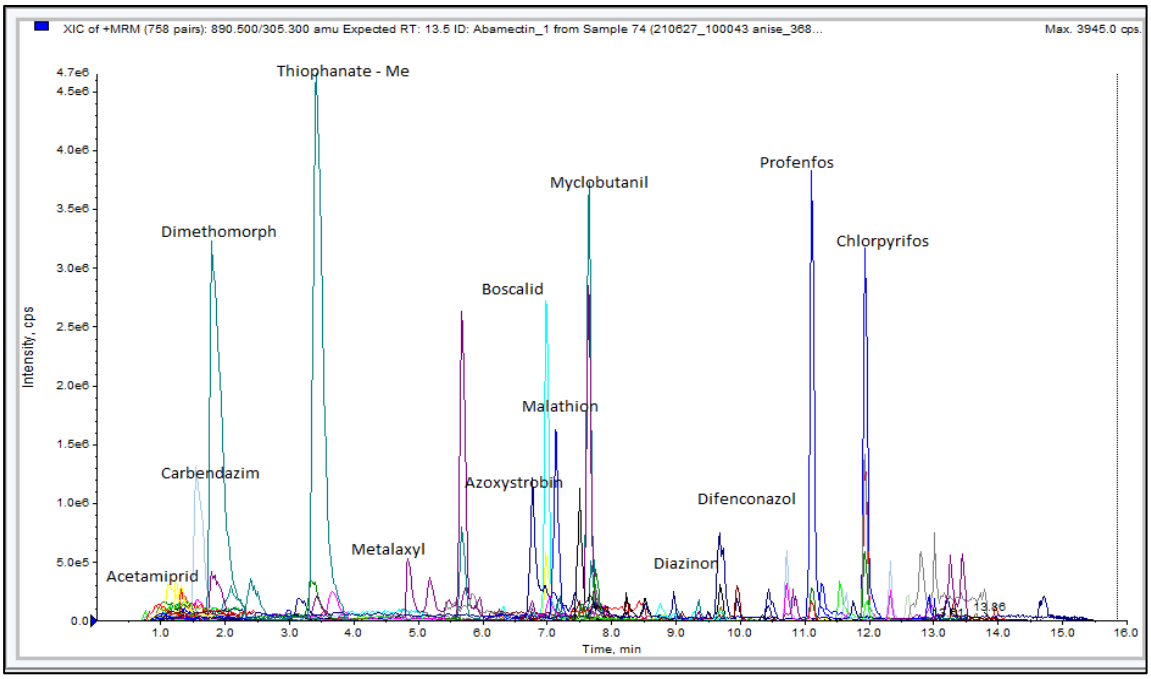

Fig. 9: LC-MS/MS Chromatogram of Anise sample No 21

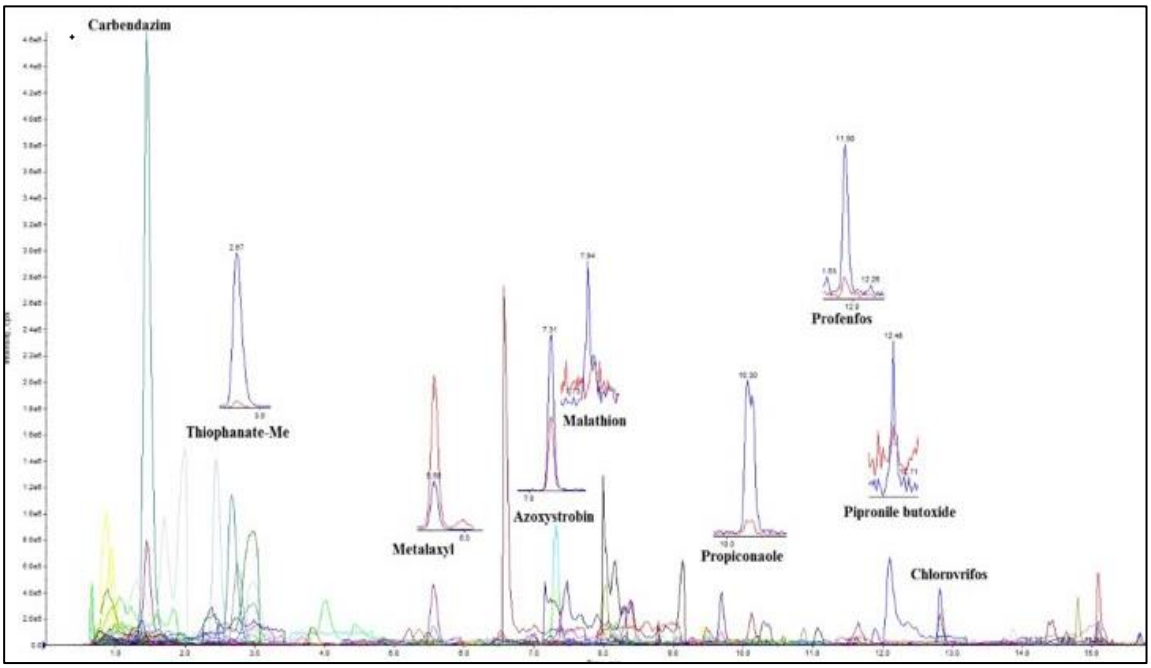

Fig. 10: LC-MS/MS Chromatogram of Chamomile sample No 23

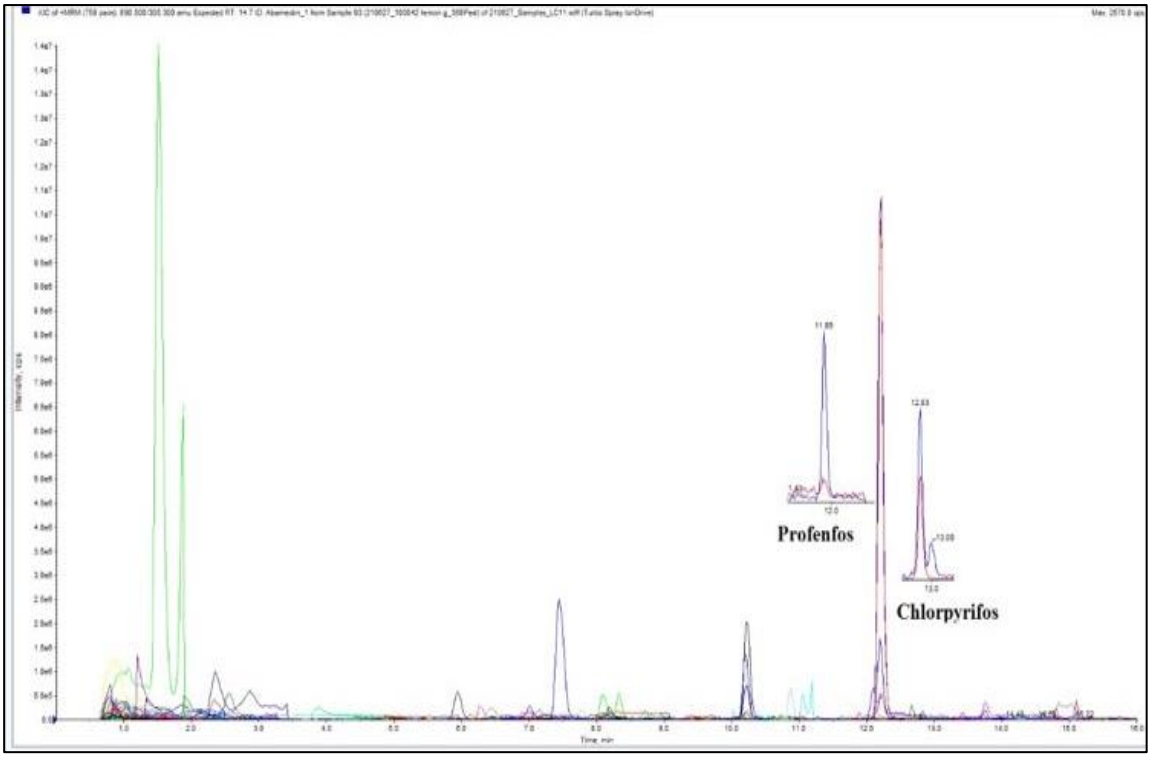

Fig. 1: LC-MS/MS chromatogram of Lemon Ginger sample No 25

2.6. Instrumentation and conditions

LC-MS/MS conditions

The LC-MS/MS analysis was conducted using an Agilent HPLC 1260 along with API 6500 QTRAP® mass spectrometer from ABSciex, connected to HPLC by electrospray ionization (ESI) interface.

The chromatographic separation of the studied pesticides was achieved using an Agilent C_{18} reverse-phase column ZORBAX® Eclipse plus 4.6 × 150 mm with 5.0 μm particle size.

The thermostatic column oven was set at 40°C, and the injection volume was 2.0 μL. A gradient elution program was used at 500 μL/min flow rate.

Reservoir A contained 10 mM ammonium formate solution in methanol: water (1: 9 v/v), and reservoir B contained LC-MS grade methanol. The total runtime was 20 min, and Analyst version 1.6 software was used for instrument control and method acquisition, as shown in Table 2.

Mass spectrometer conditions:

Multiple reactions monitoring (MRM) scan mode was used. The ESI was operated in the positive mode ESI by using the following operating parameters: the source temperature (TEM) was 450.0°C, ion spray (IS) voltage was 5000.0 V, collision gas (CAD) was set at medium, and curtain gas (CUR) was set at 30 PSI.

3. Results and Discussion

3.1. LC-MS/MS

Different gradient elution systems were tested to obtain good separation and resolution of the analyzed 34 pesticides. The best chromatographic separation was obtained using LC/MS/MS conditions shown in Table 2.

3.2. Method validation

Validation protocol is described as follows: The selected parameters for in-house validation were mainly taken from Eurachem guideline for method validation. The acceptance criteria were taken from the Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.

3.2.1. Limit of detection and limit of quantitation

The limit of detection (LOD) is defined as the lowest concentration of the analyte that can be reliably detected. In this study LOD was (0.003 mg/kg). The LOQ is the minimum concentration of analytes in the test sample that can be determined with acceptable precision (repeatability) and recovery under the stated conditions of the test. The lowest practical LOQ was estimated by using repeated spiked samples at about the expected lowest quantitation level (0.01 mg/kg) on different tea bags as shown in Table 3.

3.2.2. Method linearity

For quantitative analysis, the range of analytes (Acetamiprid, Atrazine, Azoxystrobin, Boscalid, Carbaryl, Carbendazim, Chlorfenapyr, Chlorpyrifos, Cyfluthrin, Cypermethrin, Diazinon, Difenoconazole, Dimethomorph, Flusilazole, Imidacloprid, Lambda Cyhalothrin, Malathion, Metalaxyl, Myclobutanil, Penconazole, Pendimethalin, Piperonyl butoxide, Profenofos, Propamocarb, Propargite, Propiconazole, Pyraclostrobin, Sulfur, Tebuconazole, Tetraconazole, Thiacloprid, Thiophanatemethyl and, Trifloxystrobin) concentrations over which the method may apply was determined. Calculations are based on six levels of calibration curve (ranged from 0.001 to 0.1 μg/ml) the calibration curves of the investigated pesticides were obtained by plotting values of the concentrations of pesticides against each corresponding peak area. The correlation coefficient was found to be greater than 0.99.

3.2.3. Recovery

The performance of the method was tested by performing six replicates of spiked blank samples at three different concentration levels (0.01, 0.05 and 0.1 mg/kg) for 34 pesticides using LC-MS/MS.

Results in Table **4** showed an overall good recovery ranged between 91% and 109 % for the 34 pesticides (Acetamiprid, Atrazine, Azoxystrobin, Boscalid, Carbaryl, Carbendazim, Chlorfenapyr, Chlorpyrifos, Cyfluthrin, Cypermethrin, Diazinon, Difenoconazole, Dimethomorph, Flusilazole, Imidacloprid, Lambda Cyhalothrin, Malathion, Metalaxyl, Myclobutanil, Penconazole, Pendimethalin, Piperonyl butoxide,, Profenofos, Propamocarb, Propargite, Propiconazole, Pyraclostrobin, Sulfur, Tebuconazole, Tetraconazole, Thiacloprid, Thiophanate Methyl and, Trifloxystrobin). The RSD was ≤ 10% as shown in Table 4.

3.3. Samples analysis

The 25 samples were analyzed according to the described method and the results are listed in tables 5 and 6. The obtained results showed that

there were 4 samples (S2), (S6), (S15) and (S16) containing only one residual pesticide which was Sulfur of which the concentration was below MRL. Moreover, 4 samples (S1), (S14), (S18) and (S19) all included two residual pesticides Carbendazim and Sulfur and the concentrations of the two pesticides were below MRL. Sample (S3) contained Carbendazim and sulfur which were both below MRL. Sample (S5) contained Chlorpyrifos in a concentration above MRL while Sulfur concentration was below MRL. In addition, there were 3 samples (S4) and (S11), and (S8) that showed the presence of four residual pesticides: Carbaryl, Cypermethrin, Imidacloprid, and Sulfur. Their concentrations were below MRL in (S4) and (S11) while in sample (S8), the concentration of Carbaryl was above MRL.

Furthermore the results showed that there were 2 samples (S24) and (S25) that showed the presence of 5 residual pesticides: Chlorpyrifos, Lambda-Cyhalothrin, Cypermethrin, Profenofos, and Sulfur. In Sample (S24), all the residual pesticides concentrations were below MRL except Chlorpyrifos and Profenofos. On the other hand, Chlorpyrifos and Lambda-cyhalothrin concentrations were above MRI in Sample (S25). Sample (S9) contained 7 residual pesticides: Chlorpyrifos, Lambda-Cyhalothrin, Cypermethrin, Malathion, Metalaxyl, Sulfur and, Acetamiprid. Chlorpyrifos, Malathion, and Acetamiprid were above MRL. Also, the samples (S7) and (S17) each contained 10 residual pesticides as shown in Table 6. In sample (S7), 6 residual pesticides (Malathion, Pendimethalin, Profenofos, Chlorpyrofos, Propiconazole, Chlorfenapyr) were above MRL. While in sample (S17), only 4 residual pesticides (Malathion, Profenofos, Chlorpyrofos, Chlorfenapyr) were above MRL. It was also found that there were 2 samples (S10) and (S20) that contained 13 residual pesticides. The concentrations of thiophenate-methyl, Cypermethrin, Malathion, pendimethalin, Penconazole, Piperonyl butoxide, and Azoxystrobin were below MRL while the concentrations of Carbendazim, Chlorpyrifos, Metalaxyl, Propiconazole Chlorfenapyr, and Profenofos were above MRL.

Finally, there were two samples (S21) and (S22) containing 30 residual pesticides as shown in table 5. The concentrations of only 9 pestiside residues (Carbendazim, thiophenate-methyl, Cypermethrin, Malathion, Profenofos, Acetamiprid, Propiconazole, Acetamiprid, and Myclobutanil) were above MRL.

Table 3: Limits of detection and quantification of each studied pesticide.

No.	Pesticide	LOQ (mg/kg)	LOD (mg/kg)	No.	Pesticide	LOQ (mg/kg)	LOD (mg/kg)
1	Acetamiprid	0.01	0.003	18	Metalaxyl	0.01	0.003
2	Atrazine	0.01	0.003	19	Myclobutanil	0.01	0.003
3	Azoxystrobin	0.01	0.003	20	Penconazole	0.01	0.003
4	Boscalid	0.01	0.003	21	Pendimethalin	0.01	0.003
5	Carbaryl	0.01	0.003	22	Phenthoate	0.01	0.003
6	Carbendazim	0.01	0.003	23	Piperonyl butoxide	0.01	0.003
7	Chlorfenapyr	0.05	0.003	24	Profenofos	0.01	0.003
8	Chlorpyrifos	0.01	0.003	25	Propamocarb	0.01	0.003
9	Cyfluthrin	0.01	0.003	26	Propargite	0.01	0.003
10	Cypermethrin	0.01	0.003	27	Propiconazol	0.01	0.003
11	Diazinon	0.01	0.003	28	Pyraclostrobin	0.01	0.003
12	Difenoconazole	0.01	0.003	29	Sulfur	0.05	0.003
13	Dimethomorph	0.01	0.003	30	Tebuconazole	0.01	0.003
14	Flusilazole	0.01	0.003	31	Tetraconazole	0.01	0.003
15	Imidacloprid	0.01	0.003	32	Thiacloprid	0.01	0.003
16	LambdaCyhalothrin	0.01	0.003	33	Thiophanatemethyl	0.01	0.003
17	Malathion	0.01	0.003	34	Trifloxystrobin	0.01	0.003

Table 4: Accuracy and Precision data for three levels concentration of the studied 34 pesticides using LC-ESI-MS/MS.

No.	Pesticide	Recovery ±RSD (0.1mg/kg)	Recovery ±RSD (0.05mg/kg)	Recovery ±RSD (0.01mg/kg)	Mean recovery
1.	Acetamiprid	99.80 ± 3.10	108.77 ± 1.40	92.21 ± 2.51	99.66
2.	Atrazine	94.34 ± 3.80	106.43 ± 4.73	97.45 ± 3.27	99.41
3.	Azoxystrobin	102.00 ± 4.60	103.21 ± 2.20	99.12 ± 3.49	101.44
4.	Boscalid	96 .87± 6.00	102.76 ± 9.51	92.21 ± 2.81	97.03
5.	Carbaryl	99.21 ± 9.20	105.87 ± 5.99	99.89 ± 8.40	101.66
6.	Carbendazim	99.30 ± 2.90	103.31 ± 2.00	98.55 ± 2.91	100.40
7.	Chlorfenapyr	101.67 ± 4.70	102.15 ± 2.50	99.32 ± 4.77	101.10
8.	Chlorpyrifos	100.89 ± 3.90	105.78 ± 1.31	99.12 ± 2.21	101.90
9.	Cyfluthrin	105.51 ± 6.20	107.91 ± 6.30	96.22 ± 5.41	103.21
10.	Cypermethrin	93.54 ± 9.67	96.23 ± 7.90	95.17 ± 5.21	94.98
11.	Diazinon	99.33 ± 8.80	103.71 ± 7.70	97.45 ± 9.00	100.16
12.	Difenoconazole	99.60 ± 7.10	105.34 ± 5.98	98.13 ± 9.20	101.00
13.	Dimethomorph	91.85 ± 8.60	108.17 ± 2.87	95.76 ± 6.10	98.59
14.	Flusilazole	100.34 ± 6.80	107.93 ± 4.55	101.82 ± 4.80	103.36
15.	Imidacloprid	92.90 ± 8.30	107.90 ± 4.93	91.12 ± 8.67	97.64
16.	LambdaCyhalothrin	91.81 ± 7.80	103.12 ± 3.31	95.21 ± 5.10	96,67
17.	Malathion	96.34 ± 3.80	100.10 ± 4.32	94.00 ± 2.74	96.81
18.	Metalaxyl	94.21 ± 8.50	106.85 ± 7.45	102.21 ± 4.52	101.10
19.	Myclobutanil	100.88 ± 4.30	106.13 ± 1.11	94.11 ± 6.71	100.37
20.	Penconazole	92.70 ± 8.50	94.90 ± 8.77	91.17 ± 8.64	92.92
21.	Pendimethalin	91.65 ± 9.80	109.00 ± 7.98	93.89 ± 5.77	98.18
22.	Phenthoate	95.41± 8.40	105.19 ± 3.60	93.33 ± 7.10	97.97
23.	Piperonyl butoxide	103.13 ± 3.60	106.16 ± 3.32	95.99 ± 1.98	101.76
24.	Profenofos	99.83 ± 8.80	103.49 ± 2.91	93.55 ± 8.31	98.97
25.	Propamocarb	97.16 ± 8.80	104.17 ± 7.22	94.56 ± 5.91	98.63
26.	Propargite	92.19 ± 6.21	107.21 ± 5.24	88.90 ± 4.21	92.77
27.	Propiconazol	92.01 ± 3.70	100.25 ± 4.80	99.93 ± 2.23	97.40
28.	Pyraclostrobin	104.16 ± 6.60	108.71 ± 2.40	107.98 ± 9.94	110.95
29.	Sulfur	96.78 ± 10.00	102.92 ± 6.50	93.67 ± 7.54	102.12
30.	Tebuconazole	97.84 ± 7.30	108.52 ± 3.50	99.91 ± 4.75	102.1
31.	Tetraconazole	94.99 ± 10.40	106.16 ± 6.41	96.70 ± 5.56	92.62
32.	Thiacloprid	96.65 ± 9.10	91.45 ± 8.21	92.45 ± 7.88	86.88
33.	Thiophanatemethyl	90.99 ± 8.90	92.81 ± 9.11	91.29 ± 9.11	85.03
34.	Trifloxystrobin	101.51 ± 9.70	98.22 ± 8.12	91.82 ± 4.70	99.85

Table 5: Concentrations of found residual pesticides in the examined products using LC-ESI-MS/MS.

Code	Product Name	Compound	Result in (mg/kg)	Maximum residual limit of studied pesticides (MRL) (mg/kg)	Above or below maximum residual limit of studied pesticides (MRL)
1	Green Tea with Mint	Carbendazim	<LOQ	0.1	< MRL
		Sulfur	1.2	150	< MRL
2	Anise	Sulfur	1.4	150	< MRL
3	Cinnamon	Carbendazim	<LOQ	0.1	< MRL
		Sulfur	1.4	150	< MRL
4	Hibiscus	Carbaryl	0.01	0.05	< MRL
		Cypermethrin	0.011	0.1	< MRL
		Imidacloprid	0.013	0.05	< MRL
		Sulfur	1	150	< MRL
5	Ginger	Chlorpyrifos	0.037	0.01	> MRL
		Sulfur	1.5	150	< MRL
6	Talia	Sulfur	1.1	150	< MRL
7	Green tea with mint	Atrazine	<LOQ	0.1	< MRL
		Chlorpyrifos	0.25	0.01	> MRL
		Cypermethrin	0.045	0.1	< MRL
		Malathion	0.01	0.002	> MRL
		Metalaxyl	<LOQ	0.005	< MRL
		Pendimethalin	0.025	0.005	> MRL
		Profenofos	0.304	0.005	> MRL
		Propiconazole	0.04	0.02	> MRL
		Sulfur	1.3	150	< MRL
		Chlorfenapyr	0.016	0.01	> MRL
8	Black tea with cardamom	Carbaryl	0.07	0.05	> MRL
		Cypermethrin	0.014	0.1	> MRL
		Imidacloprid	0.012	0.05	< MRL
		Sulfur	1.2	150	< MRL
9	Talia Guava	Chlorpyrifos	0.016	0.01	> MRL
		Lambda-Cyhalothrin	0.052	0.1	< MRL
		Cypermethrin	0.025	0.1	< MRL
		Malathion	0.014	0.002	> MRL
		Metalaxyl	<LOQ	0.005	< MRL
		Sulfur	0.5	150	< MRL
		Acetamiprid	0.06	0.05	> MRL
10	Chamomile	Carbendazim	0.18	0.1	> MRL
		Thiophanate-methyl	0.08	0.1	< MRL
		Chlorpyrifos	0.05	0.01	> MRL
		Cypermethrin	<LOQ	0.1	< MRL
		Malathion	<LOQ	0.002	< MRL
		Metalaxyl	0.013	0.005	> MRL
		Pendimethalin	<LOQ	0.005	< MRL
		Profenofos	0.012	0.005	> MRL
		Propiconazole	0.029	0.02	> MRL
		Chlorfenapyr	0.013	0.01	> MRL
		Azoxystrobin	0.021	0.05	< MRL
		Penconazole	<LOQ	0.05	< MRL
		Piperonyl butoxide	0.012	0.05	< MRL
11	Black tea	Carbaryl	0.04	0.05	< MRL

		Cypermethrin	0.012	0.1	< MRL
		Imidacloprid	0.013	0.05	< MRL
		Sulfur	1.1	150	< MRL
12	Black Tea	Thiacloprid	<LOQ	0.05	< MRL
13	Mint	Carbendazim	0.19	0.1	> MRL
		Thiophanate-methyl	0.07	0.1	< MRL
		Chlorpyrifos	0.06	0.01	> MRL
		Cypermethrin	<LOQ	0.1	< MRL
		Malathion	<LOQ	0.002	< MRL
		Metalaxyl	0.014	0.005	> MRL
		Pendimethalin	<LOQ	0.005	< MRL
		Profenofos	0.013	0.005	> MRL
		Propiconazole	0.027	0.02	> MRL
		Chlorfenapyr	0.015	0.01	> MRL
		Azoxystrobin	0.020	0.03	< MRL
		Penconazole	<LOQ	0.05	< MRL
		Piperonyl butoxide	0.013	0.05	< MRL
14	Tea with mint	Carbendazim	<LOQ	0.1	< MRL
		Sulfur	1.5	150	< MRL
15	Cinnamon	Sulfur	1.4	150	< MRL
16	Hibiscus	Sulfur	1.7	150	< MRL
17	Dry Mint	Atrazine	<LOQ	0.1	< MRL
		Chlorpyrifos	0.21	0.01	> MRL
		Cypermethrin	0.041	0.1	< MRL
		Malathion	0.03	0.02	> MRL
		Metalaxyl	<LOQ	0,05	< MRL
		Pendimethalin	0.028	0.05	< MRL
		Profenofos	0.301	0.05	> MRL
		Propiconazole	0.01	0.02	< MRL
		Sulfur	1.5	150	< MRL
		Chlorfenapyr	0.019	0.01	> MRL
18	Caraway	Carbendazim	<LOQ	0.1	< MRL
		Sulfur	1.1	150	< MRL
19	Green Tea	Carbendazim	<LOQ	0.1	< MRL
		Sulfur	1.2	0.1	> MRL
20	Cinnamon	Carbendazim	0.19	0.1	> MRL
		Thiophanate-methyl	0.07	0.1	< MRL
		Chlorpyrifos	0.06	0.01	> MRL
		Cypermethrin	<LOQ	0.1	< MRL
		Malathion	<LOQ	0.02	< MRL
		Metalaxyl	0.014	0.005	> MRL
		Pendimethalin	<LOQ	0.005	< MRL
		Profenofos	0.013	0.005	> MRL
		Propiconazole	0.027	0.002	> MRL
		Chlorfenapyr	0.015	0.01	> MRL
		Azoxystrobin	0.020	0.03	< MRL
		Penconazole	<LOQ	0.05	< MRL
		Piperonyl butoxide	0.013	0.05	< MRL
21	Anise	Carbendazim	0.15	0.1	> MRL
		Thiophanate-methyl	0.208	0.1	> MRL
		Chlorpyrifos	0.75	0.01	> MRL
		Cyfluthrin	0.016	0.1	< MRL
		Lambda-Cyhalothrin	0.051	0.1	< MRL
		Cypermethrin	0.11	0.1	> MRL
		Diazinon	0.015	0.05	< MRL
		Malathion	0.54	0.02	> MRL

		Metalaxyl	0.03	0.05	< MRL
		Pendimethalin	0.026	0.05	< MRL
		Profenofos	0.5	0.05	> MRL
		Propargite	0.012	0.05	< MRL
		Propiconazole	0.19	0.02	> MRL
		Tebuconazole	0.022	0.05	< MRL
		Imidacloprid	0.048	0.05	< MRL
		Phenthoate	<LOQ	0.05	< MRL
		Difenoconazole	0.037	0.05	< MRL
		Acetamiprid	0.092	0.05	> MRL
		Flusilazole	<LOQ	0.05	< MRL
		Azoxystrobin	0.018	0.03	< MRL
		Trifloxystrobin	0.019	0.05	< MRL
		Penconazole	0.023	0.05	< MRL
		Propamocarb	0.01	0.05	< MRL
		Dimethomorph	0.016	0.05	< MRL
		Piperonyl butoxide	<LOQ	0.05	< MRL
		Myclobutanil	0.039	0.05	< MRL
		Boscalid	0.01	0.9	< MRL
		Pyraclostrobin	0.01	0.1	< MRL
		Tetraconazole	<LOQ	0.02	< MRL
22	Hibiscus	Carbendazim	0.17	0.1	> MRL
		Thiophanate-methyl	0.205	0.1	> MRL
		Chlorpyrifos	0.78	0.01	> MRL
		Cyfluthrin	0.018	0.1	< MRL
		Lambda-Cyhalothrin	0.050	0.1	< MRL
		Cypermethrin	0.15	0.1	> MRL
		Diazinon	0.019	0.05	< MRL
		Malathion	0.50	0.02	> MRL
		Metalaxyl	0.09	0.005	> MRL
		Pendimethalin	0.022	0.005	> MRL
		Profenofos	0.8	0.005	> MRL
		Propargite	0.016	0.05	< MRL
		Propiconazole	0.16	0.002	> MRL
		Tebuconazole	0.024	0.05	< MRL
		Imidacloprid	0.049	0.05	< MRL
		Phenthoate	<LOQ	0.05	< MRL
		Difenoconazole	0.039	0.05	< MRL
		Acetamiprid	0.090	0.05	> MRL
		Flusilazole	<LOQ	0.05	< MRL
		Azoxystrobin	0.017	0.05	< MRL
		Trifloxystrobin	0.016	0.03	< MRL
		Penconazole	0.025	0.05	< MRL
		Propamocarb	0.04	0.05	< MRL
		Dimethomorph	0.015	0.05	< MRL
		Piperonyl butoxide	<LOQ	0.05	< MRL
		Myclobutanil	0.039	0.05	< MRL
		Boscalid	0.01	0.05	< MRL
		Pyraclostrobin	0.04	0.9	< MRL
		Tetraconazole	<LOQ	0.1	< MRL
23	Chamomile	Carbendazim	0.18	0.1	> MRL
		Thiophanate-methyl	0.08	0.1	< MRL
		Chlorpyrifos	0.05	0.01	> MRL
		Cypermethrin	<LOQ	0.1	< MRL
		Malathion	<LOQ	0.02	< MRL
		Metalaxyl	0.013	0.005	> MRL

		Pendimethalin	<LOQ	0.005	< MRL
		Profenofos	0.012	0.005	> MRL
		Propiconazole	0.029	0.002	> MRL
		Chlorfenapyr	0.013	0.01	> MRL
		Azoxystrobin	0.021	0.03	< MRL
		Penconazole	<LOQ	0.05	< MRL
		Piperonyl butoxide	0.012	0.05	< MRL
24	Green Tea	Chlorpyrifos	0.025	0.01	> MRL
		Lambda-Cyhalothrin	0.026	0.1	< MRL
		Cypermethrin	0.024	0.1	< MRL
		Profenofos	0.028	0.002	> MRL
		Sulfur	2.3	150	< MRL
25	Lemon with Ginger	Chlorpyrifos	0.027	0.01	> MRL
		Lambda-Cyhalothrin	0.024	0.01	> MRL
		Cypermethrin	0.023	0.1	< MRL
		Profenofos	0.026	0.05	< MRL
		Sulfur	2.1	150	< MRL

Conclusion:

The obtained results showed that about 34 pesticides have been determinded in most of the examined samples. Limit of quantitation for the studied pesticides equals (0.01 mg/kg). The developed method was employed for the analysis of studied residual pesticides in real tea bags samples collected from different companies in the Egyptian market. The obtained results showed that most of the collected samples showed the presence of different pesticide residues, some of them exceeding MRL which indicates the presence of uncontrolled pesticide practices.

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