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RESEARCH ARTICLE

Investigation of pesticide residues level on commonly consumed leafy vegetables picked from the central market in Jeddah, Saudi Arabia

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Abstract

This study aimed to investigate the presence of pesticide residues in a variety of commonly consumed leafy vegetables, including Grape leaves, Lettuce, Arugula, Spinach, Purslane, Ocimum, Parsley, Jew's mallow, Celery, Coriander, and Mint. A total of 100 samples were collected from the Central Market of Jeddah, Kingdom of Saudi Arabia. Our methodology involved employing the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction method in combination with Liquid Chromatography-Tandem Mass Spectrometry (LC–MS/MS) to analyze a comprehensive database of 237 distinct pesticides. The range for limit of detection (LOD) and limit of quantification (LOQ) of the method were 0.0001 to 0.0014 mg. Kg^{-1} and 0.0010 to 0.0064 mg. Kg^{-1} for tested pesticides, respectively. The recoveries were in the range of 70–172.9%, with a relative standard deviation (RSD) of less than 19.0% for all tested pesticides. The results revealed that 60% of the analyzed samples were free from pesticide residues, while 40% exhibited contamination with 17 different pesticide residues. Notably, the most prevalent pesticide detected was Triallate in the Ocimum samples, followed by Metalaxyl in Grape leaves, Mint, and Spinach, and Methomyl in Celery. Approximately 45% of the samples contained pesticide residues that fell below or were equal to the European Union Maximum Residue Levels (EU MRLs), while the remaining 55% exceeded these MRLs. Remarkably, high pesticide concentrations were observed in all Ocimum samples (Triallate, Pyridaben, Hexythiazox, Imidacloprid), 67% of Grape leaves (Metalaxyl, Azoxystrobin, Difenoconazole Isomer), and 40% of Celery (Azoxystrobin, Methomyl). In conclusion, this study sheds light on the contamination levels of commonly consumed domestically produced and purchased leafy vegetables in the Central Market of Jeddah. To ensure food safety and the well-being of consumers, we strongly recommend enhanced scientific assessments and continued monitoring of pesticide usage in agricultural practices.

Keywords Contamination · MRL · Vegetables · QuEChERS · Food safety

Introduction

Pesticides are defined as groups of substances, either chemical or biological in nature, which differ in their physical, chemical, and identical properties from one to another. They are employed to repel, destroy, or control pests and contribute to improved crop yields and decreased post-harvest losses (Food and Agriculture Organization (FAO), 2022).

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Despite their vital role, concerns have arisen about the long-term presence of these pesticide residues in leafy vegetables and the potential risks they pose to human health (Kalyabina et al. 2021). The accumulation of pesticide residues in agricultural crops raises concerns about potential acute or chronic toxicity to consumers' health. Also, the low levels of pesticide exposure can have adverse effects on infant neural and social development, intellectual development, and the occurrence of deficit hyperactivity disorder. Moreover, potential health effects include hepatotoxicity, genotoxicity, and various types of cancer (Liu and Schelar 2012; Alavanja et al. 2013; Park et al. 2021).

Globally, there are approximately 1400 pesticides in use. Most of them have been designed to selectively combat specific pathogens based on the physiological activities of the target pathogen (Turinek et al. 2009). They are generally classified into insecticides (for killing insects and other arthropods), herbicides (for eliminating unwanted weeds), fungicides (for combating fungi), or rodenticides (for controlling mice and other rodents) (Sarkar et al. 2021). World Health Organization (WHO) classes and identifies pesticide residues in food from I (very high toxicity) to III (low toxicity) (WHO 2010).

In Saudi Arabia, there has been a significant increase in pesticide usage and agriculture development (Saggu et al. 2016). Recent research conducted in various regions of Saudi Arabia has focused on pesticide residue analysis, revealing the persistence of multiple pesticides in residual amounts across various commodities (Osman et al. 2010; Saggu et al. 2016). Maximum Residue Limits (MRLs) are widely employed to regulate pesticide residues. MRLs represent the maximum permissible levels of pesticide residues in food when pesticides are used following accepted agricultural practices (EL-Saeid and Selim 2013; Crépet et al. 2021). These levels are set below concentrations considered harmful to humans, serving as a global standard for managing pesticide levels in food (Ambrus and Yang 2016).

Green leafy vegetables are necessary for human nutrition, providing essential nutrients required for numerous biochemical reactions and metabolic pathways within the body (Amer et al. 2018; Arif et al. 2020; Houngla et al. 2020; Mohamed et al. 2023). A diet rich in green leafy vegetables is crucial for maintaining normal bodily functions, preventing vitamin deficiencies, and reducing the risk of serious diseases (Sarwar et al. 2021). Like other crops, vegetables are susceptible to pests and pathogens attacks during their production and storage, which can negatively impact their quality and yield. Unlike grains, most vegetables are typically consumed raw, boiled, or steamed, making the impact of their health qualities on consumers relatively more significant. Additionally, green leafy vegetables often retain pesticide residues due to their relatively large surface area (The European Food Safety Authority (EFSA) 2008).

It is noteworthy that, thus far, there has been a significant lack of data regarding pesticide residues in leafy vegetables in the western region of Saudi Arabia, specifically in Jeddah city. Against this backdrop, this study aimed to detect and quantify pesticide residue levels in commonly consumed leafy vegetables domestically produced and available in the Central Market in Jeddah city, KSA. Additionally, the pesticide concentrations were assessed to determine compliance with European Union Maximum Residue Levels (EU MRLs), serving as a reference for future monitoring endeavors.

Materials and methods

Sample collection

This study was performed in the Food Control Lab, Jeddah, KSA. One hundred samples of leafy vegetables were collected from the Central Market of Fruit and Vegetables in Jeddah city from 21 April to 27 June 2022. The selected samples were from 11 different types of leafy vegetables as follows: Grape leave (*Vitis vinifera*); Lettuce (*Lactuca sativa*); Arugula (*Eruca vesicaria*); Spinach (*Spinacia oleracea*); Purslane (*Portulaca oleracea*); Ocimum (*Ocimum basilicum*); Parsley (*Petroselinum crispum*); Jew's mallow (*Corchorus olitorius*); Celery (*Apium graveolens*); Coriander (*Coriandrum sativum*); Mint (*Mentha*). The selection of these leafy vegetables was based on their high consumption rates. All samples were placed in polythene bags labeled by cultivation place and time of purchase.

Sample preparation

On the first day of the experiment, 100g of each sample was weighed, then chopped and thoroughly blended with a food blender, and 100 ml of distilled water was added to optimize the extraction. The homogenized samples were put into plastic bags and frozen immediately to minimize the risk of degradation of pesticide residues present. Samples were stored at 4ºC in the refrigerator for extraction and analysis. One hundred samples were collected from 11 different types of leafy vegetables, including Arugula (12 samples), Celery (5 samples), Coriander (10 samples), Grape leaves (3 samples), Jew's Mallow (12 samples), Lettuce (10 samples),2 Mint (11 samples), Ocimum (7 samples), Parsley (11 samples), Purslane (8 samples), and Spinach (11 samples). These vegetables were sourced from various regions, including Makkah, Tabuk, Hail, Al Madinah, and other neighboring areas surrounding Jeddah city. Each type of leafy vegetable was selected from its respective cultivation area. The Jeddah Central Market for Vegetables served as the central hub for collecting these samples, as illustrated in Fig. 1.

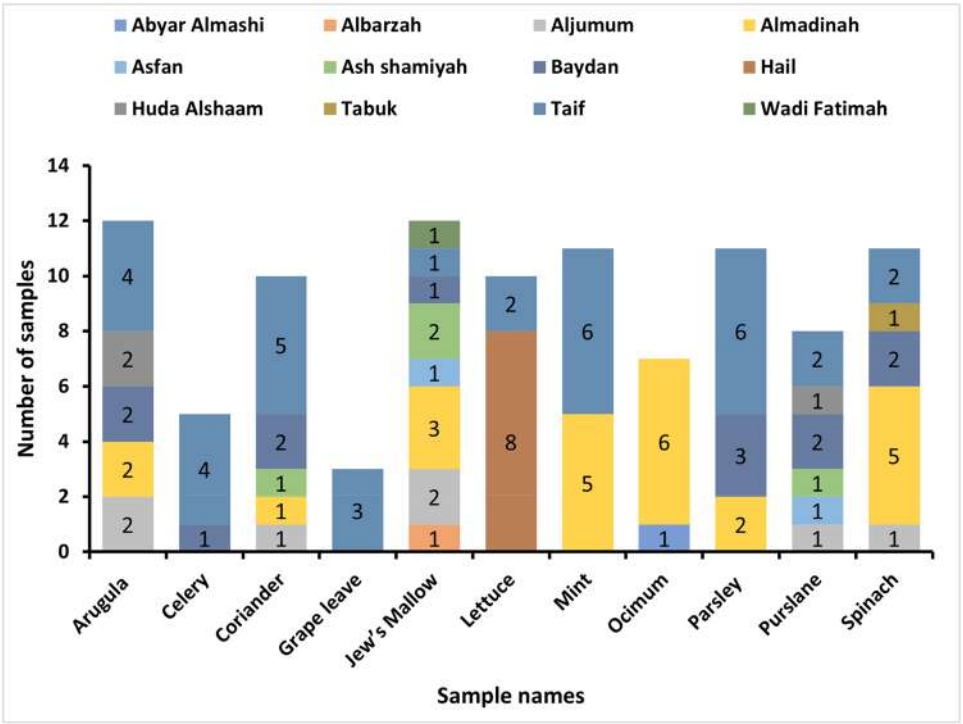


Fig. 1 Numbers and names of leafy vegetable samples collected from Jeddah City. One hundred samples were collected from 11 different types of leafy vegetables: Arugula (12), Celery (5), Coriander (10), Grape leaves (3), Jew's Mallow (12), Lettuce (10), Mint (11), Ocimum (7), Parsley (11), Purslane (8), and Spinach (11)

Extraction procedure

The extraction process was carried out using the Quick, Easy, Cheap, Efficient, Robust, and Safe (QuEChERS) method, as described by (Anastassiades et al. 2003). For this purpose, we employed a QuEChERS kit (Agilent, Part Number 5982–5650), followed by a dispersed solid-phase extraction (d-SPE) using a 2 ml (Agilent, Part Number 5982–5021). First, 10 g of each homogenized sample were weighed and placed into separate 50 mL centrifuge tubes. Subsequently, 10 mL of acetonitrile (ACN) with a purity level of 99.9% (ROMIL) was added to each sample. To ensure thorough mixing, the samples were then subjected to agitation in a Geno/Grinder, operating at 1240 revolutions per minute (rpm), for 2 min. The samples were separated into two layers following the mixing step, with the upper layer containing the ACN solvent. This choice of ACN as the extraction solvent was based on its demonstrated superiority over ethyl acetate (EtOAc) and acetone, as highlighted by (Lehotay 2011).

We proceeded by opening the centrifuge tubes and introducing the QuEChERS extraction salts and buffers kit, which included the following components: Magnesium sulfate ($MgSO_4$) with a total sorbent weight of 4000 mg per tube, sodium chloride (NaCl) with a total sorbent weight of 1000 mg per tube, sodium citrate dihydrate with a total sorbent weight of 1000 mg per tube, and sodium citrate sesquihydrate with a total sorbent weight of 500 mg per tube. $MgSO_4$ and NaCl were incorporated to eliminate residual water from the acetonitrile layer, while sodium citrate dihydrate and sodium citrate sesquihydrate served as buffering agents.

Subsequently, we sealed the tubes and subjected them to an additional 2 min of shaking at 1240 rpm in the Geno/Grinder. This step facilitated the extraction process, particularly for non-polar pesticides, owing to the heating effect resulting from the exothermic hydration of $MgSO_4$. Finally, the samples underwent centrifugation at 4600 rpm for 5 min, separating layers. The supernatant containing the extracted pesticide residues was carefully collected for further purification.

Sample Clean-up

The extracted pesticide residues are washed to remove any interfering substances that may have been co-extracted with them. This step is crucial to achieve the high sensitivity required for the subsequent analysis. By adding the (ACN) layer extract to a combination containing 150 mg ($MgSO_4$), 25 mg primary-secondary amine (PSA) sorbent, and 7.5 mg graphitized carbon black (GCB.PSA) itself and/or the GCB/PSA bilayer, which has proven to be the most effective for sample purification.

Next, the tube was vigorously shaken for 1 min using a Thermomixer at 1500 RPM and then centrifuged at 1000 RPM for 5 min. Subsequently, 100 µL of the supernatant was collected and diluted with 900 µL of mobile phase (a mixture of Water and Metanol) in autosampler vials. The samples were then vortexed for 30 s utilizing a Genie vortex. Finally, the extract 5µl was used for liquid Chromatography-Tandem Mass Spectrometry (LC–MS/MS) analysis.

LC–MS/MS analysis

The analysis was conducted using an ESI-LC–MS/MS Triple Quad instrument (Sciex 6500, Germany) in conjunction with a 1290 Infinity LC system (Agilent, Waldbronn, Germany). The LC–MS/MS setup was performed according to the Food Control Lab Reference Method for analyzing pesticides in leafy vegetables.

Chromatographic separation was meticulously performed on a Zorbax Eclipse Plus C18 reversed-phase column with the following specifications: 2.1×150 mm dimensions, a particle size of 1.8 µm, and a pore size of 9.5 nm, all sourced from Agilent in Waldbronn, Germany. The injection volume was precisely set at 5 µl. The LC system operated with mobile phase A, comprised of water, and mobile phase B, consisting of methanol. Both mobile phases contained a 5 mM ammonium format solution and 1% formic acid.

Nitrogen was employed as the curtain gas, ensuring optimal performance. MS/MS conditions were meticulously optimized through direct infusion into the electrospray ionization source (ESI) in positive mode, aiming to provide the highest signal-to-noise ratio for quantified ions for each analyte. The ion spray potential was set at 5500 V. In contrast, the input potential was maintained at 10 V. Each run took approximately 25 min for the samples to elute, demonstrating the thoroughness and precision of the analytical procedure.

The LC–MS/MS method performance was estimated by evaluating some quality parameters, such as the recovery values, precision, limits of quantification (LOQ), and limits of detection (LOD). RSD (a relative standard deviation) was used for the assessment of intra-day precision and inter-day precision (Fan et al. 2022). LOQ and LOD were evaluated according to the following equations described by Thomsen et al. (2003):

$$LOQ = 10S_0/b, \text{and} LOD = 3.3S_0/b$$

where S_0 is the calibration line standard deviation and b is the slope.

The linearity was significant with an excellent correlation coefficient of $R = 0.998$. The ranges for LOQ and LOD were 0.0001 to 0.0014 mg. Kg^{-1} and 0.0010 to 0.0064 mg. Kg^{-1} for tested pesticides, respectively (Table 2). The recoveries of tested pesticides at different levels of fortification, i.e., 0.01, 0.1, and 1.0 mg Kg^{-1}, were estimated in leafy vegetable samples. Under the selected conditions, we did not observe any interfering peaks on the sample chromatogram. The recoveries were in the range of 70–172.9%, with a relative standard deviation (RSD) of less than 19.0% for all tested pesticides (Table 3).

Statistical analysis

The recovery data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 18.0) program with Duncan's Multiple Range Test with a $p < 0.05$ significance value to determine statistical differences of recoveries among different concentrations within each product type.

Results

Evaluation by commodity

Pesticide residues were identified in all the collected samples with varying concentrations. Our results detected the concentration of 17 different pesticides that were determined in 10 types of leafy vegetables. The descriptive results presented in Table 1 revealed that all coriander samples were free from pesticide residues. Also, 60% of the samples showed no detectable pesticide residues. Among the contaminated samples, 18 of them (representing 45%) contained a detectable level of pesticides below the (EU MRLs) (European Union 2023). However, 22 samples (55% of the contaminated samples) contained pesticide residues that exceeded the (EU MRLs) As shown in Fig. 2.

Evaluation by pesticide

Table 2 provides a list of detected pesticides in each sample and a summary of the descriptive statistics concerning the concentration values of detected pesticides that exceeded the (EU MRLs) in the analyzed leafy vegetable samples. Pesticide residues exceeding the EU MRLs were identified in Celery, Grape leaves, Mint, Spinach, Parsley, and Ocimum. Notably, the highest levels of pesticide residues were observed in Ocimum, Grape leaves, and Celery. Among these pesticides, Triallate was the most frequently detected residue, with a concentration of 0.154 ± 0.005 mg/kg recorded only in Ocimum samples. Metalaxyl was the second most prevalent, with concentrations of 0.024 ± 0.003 mg/kg, 3.069 ± 0.043 mg/kg, and 25.01 ± 2.985 mg/kg detected in the tested samples, including Spinach, Mint, and Grape leaves.

Additionally, Celery exhibited the highest recorded concentration among all detected pesticides for Methomyl, with a concentration of 10.926 mg/kg. Furthermore, the results exhibited high pesticide residue percentages in Ocimum (100%), Parsley (72%), and Grape leaves (66.7%), moderate percentages in Spinach (54%), Mint (45%), and Jew's Mallow (41.9%), and lower percentages in Celery (33.3%), Arugula (16.6%), Purslane (14%), and Lettuce (10%).

Figure 3 illustrates that Al Madinah exhibited the highest percentage of leafy vegetables, exceeding the MRLs,

Table 1 Frequency of samples with and without detectable pesticides residues and samples containing residues above EU MRL for leafy vegetables

Leafy vegetables	No of the samples analyzed	No of samples without detectable residues (%)	No of samples ≤ EU MRL (%)	No of samples > EU MRL (%)
Arugula	12	10 (83.3%)	2 (17%)	0 (0%)
Celery	5	2 (40%)	1 (20%)	2 (40%)
Coriander	10	10 (100%)	0 (0%)	0 (0%)
Grape leaves	3	1 (33%)	0 (0%)	2 (67%)
Jew's Mellow	12	7 (58%)	4 (33%)	1 (8%)
Lettuce	10	9 (90%)	1 (10%)	0 (0%)
Mint	11	6 (55%)	2 (18%)	3(27%)
Ocimum	7	0 (0%)	0 (0%)	7 (100%)
Parsley	11	3 (27%)	4 (36%)	4 (36%)
Purslane	8	7 (87%)	0 (0%)	1 (13%)
Spinach	11	5 (46%)	4 (36%)	2 (18%)
Total	100 (100%)	60 (60%)	18 (45%)	22 (55%)

EU MRL: European Union Maximum Residue Levels

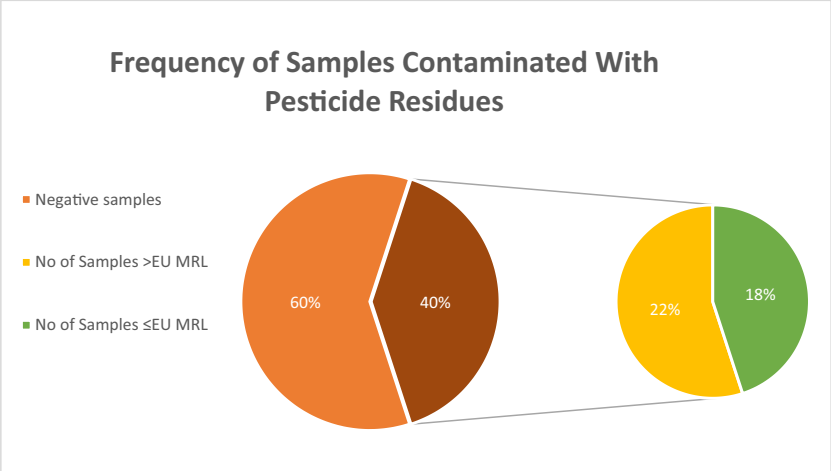


Fig. 2 Frequency of samples contaminated with pesticide residues above and below the EU MRL. This chart draw by R programming (Statistical Programming Language)

with approximately 59% (including Mint, Ocimum, Parsley, and Spinach). Following Al Madinah, the Makkah region had 36% of the leafy vegetables surpassing the MRLs, including Celery, Grape leaves, Jew's Mallow, Mint, Purslane, and Parsley. In contrast, the lowest percentage of vegetables exceeding the MRLs, just 5%, was observed in Tabuk (specifically Spinach) (Table 3).

Discussion

Several scientific studies have been conducted to detect and quantify pesticide residue levels in fruit markets across Saudi Arabia (Abd-Elhaleem 2020; Khatri et al. 2020). A comprehensive case study conducted in Jeddah, KSA, demonstrated a concerning prevalence of cypermethrin pesticide residues in vegetables, which is classified as a potential human carcinogen (Mosleh et al. 2014). Extensive research efforts have consistently highlighted that consuming fruits and vegetables contaminated with pesticides is the primary avenue of exposure for the general population (Reeves et al. 2019; Park et al. 2021).

Similarly, Osman et al. (2010) conducted a rigorous analysis of pesticide residues in vegetables available in the Al-Qassim region. Their findings were rather alarming, with 55% of the samples demonstrating the presence of pesticide residues. Even more concerning, 60% of these contaminated samples surpassed the MRLs, indicating a substantial health risk. Notably, many pesticides can impact human health, even at low concentrations, as repeated consumption of contaminated vegetables can lead to the accumulation of these pesticides in the body (Kalyabina et al. 2021).

Table 2 Sample names, detected pesticides, LOD, and LOQ and mean values compared to EU MRLs

Sample	Pesticide	Mean ± SD (mg. Kg^{-1})	No. and % of samples exceed EU MRLs	LOD (mg. Kg^{-1})	LOQ (mg. Kg^{-1})	EU MRLs (mg Kg^{-1})
Jew's Mellow	Indoxacarb	4.494 ± 3.132	1(8%)	0.0004	0.0018	3
Purslane	Imidacloprid	0.594 ± 0.025	1(13%)	0.0001	0.0050	0.01
Parsley	Acetamiprid	0.257 ± 0.021	4(36%)	0.0001	0.0010	0.01
	Penconazole	0.895 ± 0.063		0.0008	0.0063	0.02
Celery	Azoxystrobin	1.567 ± 0.6386	2 (40%)	0.0013	0.0040	0.3
	Difenoconazole Isomer	0.519 ± 0.320		0.0007	0.0049	0.3
	Difenoconazole Isomer2	0.485 ± 0.169		0.0014	0.0059	0.3
	Methomyl	10.926 ± 1.489		0.0001	0.0012	0.05
Ocimum	Triallate	0.154 ± 0.005	7(100%)	0.0019	0.0064	*
	Pyridaben	6.258 ± 0.472		0.0005	0.0043	0.02
	Hexythiazox	0.561 ± 0.023		0.0008	0.0051	0.02
	Imidacloprid	0.304 ± 0.021		0.0001	0.0050	0.05
Mint	Imidacloprid	0.408 ± 0.020	3(27%)	0.0001	0.0050	0.05
	Pyridaben	0.209 ± 0.034		0.0005	0.0043	0.2
	Hexythiazox	0.338 ± 0.015		0.0008	0.0051	0.02
	Quinalphos	0.181 ± 0.003		0.0001	0.0039	0.02
	Metalaxyl	3.069 ± 0.043		0.0007	0.0047	3
Spinach	Buprofezin	0.048 ± 0.003	2(18%)	0.0001	0.0035	0.01
	Difenoconazole Isomer	1.080 ± 0.065		0.0007	0.0049	0.3
	Difenoconazole Isomer2	1.016 ± 0.071		0.0014	0.0059	0.3
	Metalaxyl	25.01 ± 2.985		0.0007	0.0047	1.5
	Quinalphos	0.0289 ± 0.004		0.0001	0.0039	0.01
Grape leaves	Metalaxyl	0.024 ± 0.003	2(67%)	0.0007	0.0047	0.01
	Azoxystrobin	3.368 ± 0.537		0.0013	0.0040	0.3
	Difenoconazole Isomer	1.605 ± 0.164		0.0007	0.0049	0.3
	Difenoconazole Isomer2	1.493 ± 0.174		0.0014	0.0059	0.3

EU MRLs weren't established to use on Ocimum, EU MRL: European Union Maximum Residue Levels, LOQ: limits of quantification, LOD: limits of detection

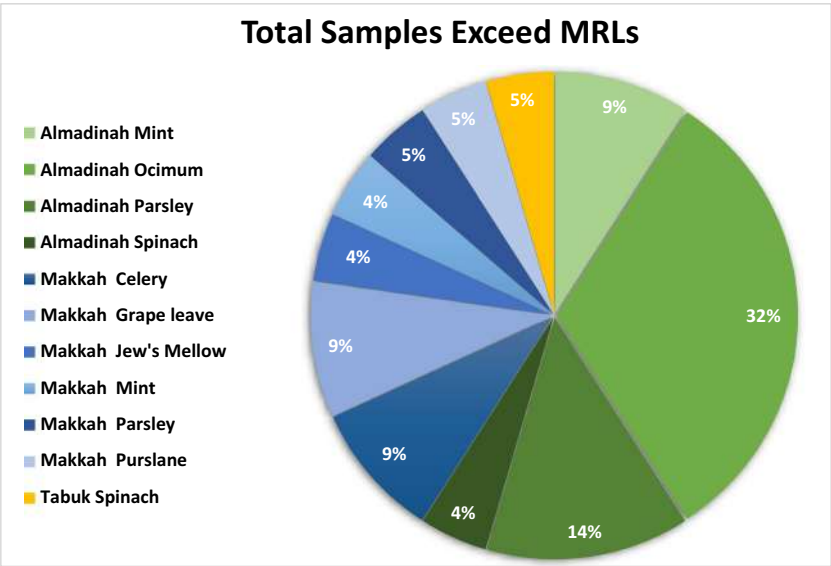


Fig. 3 Percentage of leafy vegetables exceeding the EU MRLs. This chart draw by R programming (Statistical Programming Language)

Table 3 Percentages of recovery of tested pesticides from leafy vegetables

Sample	Pesticides	Spiked Concentrations					
		Average recovery %			RSD		
		0.01 (mg kg^{-1})	0.1 (mg kg^{-1})	1 (mg kg^{-1})	0.01 (mg kg^{-1})	0.1 (mg kg^{-1})	1 (mg kg^{-1})
Jew's Mellow	Indoxacarb	78.0^{c}	86.0	91.0^{d}	7.1	4.9	5.9
Purslane	Imidacloprid	75.0^{d}	89.0^{d}	94.0^{c}	14.1	1.4	10.5
Parsley	Acetamiprid	72.0^{e}	81.5^{f}	89.0^{e}	7.3	2.5	7.9
	Penconazole	82.0^{c}	89.0^{d}	96.5^{c}	5.0	1.0	6.1
Celery	Azoxystrobin	84.0^{c}	91.0^{d}	95.0^{c}	7.0	2.0	9.0
	Difenoconazole Isomer	73.5^{e}	79.0^{f}	84.0^{f}	9.3	4.0	12.0
	Difenoconazole Isomer2	77.0^{d}	86.0^{e}	90.5^{e}	7.6	18.0	13.4
	Methomyl	94.5^{a}	109.5^{b}	106.7^{b}	15.3	4.0	8.0
Ocimum	Triallate	82.5^{c}	93.1^{d}	103.2^{b}	13.1	9.0	3.1
	Pyridaben	80.0^{c}	87.4^{e}	94.0^{c}	6.0	12.0	16.0
	Hexythiazox	81.7^{c}	88.4^{e}	93.1^{d}	6.4	1.4	4.6
	Imidacloprid	73.0^{e}	86.2^{e}	92.6^{d}	3.5	3.5	6.5
Mint	Imidacloprid	77.5^{d}	86.8^{e}	95.3^{c}	6.5	4.0	4.5
	Pyridaben	87.9^{b}	110.0^{b}	125.6^{a}	1.4	19.6	6.1
	Hexythiazox	72.5^{e}	88.0^{e}	84.5^{f}	10.5	10.5	2.6
	Quinalphos	89.0^{b}	118.0^{a}	127.9^{a}	8.6	3.2	5.0
	Metalaxyl	67.5^{f}	74.8^{g}	89.3^{e}	17.0	8.0	3.0
Spinach	Buprofezin	88.3^{b}	97.1^{c}	124.9^{a}	7.2	15.0	16.7
	Difenoconazole Isomer	70.0^{f}	83.6^{f}	91.4^{d}	4.9	3.5	10.9
	Difenoconazole Isomer2	73.0^{e}	86.1^{e}	96.4^{c}	3.5	13.4	8.3
	Metalaxyl	89.0^{b}	118.0^{a}	127.6^{a}	1.4	19.0	7.6
	Quinalphos	79.1^{c}	88.4^{e}	95.3^{c}	16.1	9.0	5.6
Grape leaves	Metalaxyl	86.1^{b}	98.5^{c}	105.0^{b}	5.7	10.0	14.2
	Azoxystrobin	76.0^{d}	86.9^{e}	93.0^{d}	14.0	7.3	2.0
	Difenoconazole Isomer	70.1^{f}	76.6^{g}	84.2^{f}	3.0	9.4	16.4
	Difenoconazole Isomer2	76.1^{d}	83.4^{f}	92.4^{d}	7.1	7.1	5.0

Average values (mg kg^{-1}) in columns for each concentration and each product type carrying different superscript letters are statistically different at $p < 0.05$ when analyzed using SPSS 18 software. RSD: relative standard deviation

Our findings indicate that Methomyl was detected in the collected samples, with a residue concentration of approximately 10.926 mg/kg, the highest value among all detected pesticides. Methomyl is an insecticide that controls pests that attack vegetables during production and storage (Grewal 2017). Methomyl was banned and restricted according to the Pesticides Law System and it is implemented and regulated in the Gulf Cooperation Council (GCC) (SFDA 2015). The United States Environmental Protection Agency (US EPA) classified methomyl as highly toxic to humans via oral exposure, which functionally affects human growth and inhibits enzyme activity, disrupting the antioxidant system in the human (Yamagishi et al. 2021; Jing et al. 2022). As reported by Chabane et al. (2022), the colon and duodenum of rats exposed to methomyl showed severe pathological changes. Furthermore, completely modified intestinal rat tissue's structure and antioxidant status might lead to intestinal and digestive dysfunctions. Even though methomyl is a banned substance and highly toxic, it is still used illegally by some farmers. This can pose a human health risk (Arias et al. 2014).

In addition, our results indicate that Triallate, Metalaxyl, and Pyridaben were the most frequently detected pesticide residues in the collected samples of leafy vegetables. Triallate is a selective herbicide to control grass weeds in the field and pulse crops, which is also classified as a Group C chemical (possible human carcinogen) (Gressel 2018). The detection of trillate in leafy vegetables indicates the importance of more scientific monitoring of the management of pesticide use in farms (Beckie et al. 2019). Metalaxyl is one of the best fungicides, and Pyridaben is a selective insecticide (Deveci et al. 2023). The detection of metalaxyl residues exceeding the MRL may be due to a lack of awareness among farmers to spray this pesticide formulation at the proper concentration (WHO 2023).

Furthermore, acetamiprid insecticide was detected in 36% of the leafy vegetable samples. As mentioned by Eltoweissy et al. (2022), acetamiprid induced oxidative damage and genotoxicity in male albino rats. The findings further support the harmful effects and the potential toxicity of acetamiprid on the male reproductive. Also, the quinalphos insecticide ratio exceeded the EU MRLs on spinach and Mint samples. The main metabolite of quinalphos is 2-hydroxyquinoline (HQO). The organophosphate quinalphos does not dispose of reactive groups capable of inducing oxidative stress directly, a secondary oxidotoxicity induced by HQO, an electron-rich molecule containing two nitrogen atoms and carrying a hydroxyl group, which should easily undergo redox reactions (Sundukov 2006).

The results revealed varying pesticide residue percentages in different vegetables. Ocimum, Parsley, and Grape leaves had high percentages. Spinach, Mint, and Jew's Mallow showed moderate percentages, while Celery, Arugula, Purslane, and Lettuce exhibited lower percentages. It was postulated that Ocimum, Parsley and Grape leaves are highly likely to be contaminated with pesticides because these leafy vegetables have broad surface areas full of zigzags compared to the other types (Farha et al. 2018). The findings of our study are consistent with other research highlighting the widespread presence of pesticide residues in vegetables. For instance, (Dinede et al. 2023) found pesticide residues in 60% of Swiss chard, 47% of cabbage, and 45% of tomato samples. The prevalence of pesticide residues in agricultural output was further highlighted by a study by (El-Sheikh et al. 2022), which revealed that pesticide residues exceeding the MRLs were found in 41% of vegetables and 39% of fruits.

These findings align with our investigation findings, which showed that different vegetables had moderate to high levels of pesticide residue. An investigation of pesticide residues in vegetables from the Asir area of Saudi Arabia undertaken by Ramadan et al. (2020) found that samples of lettuce, cauliflower, and carrots revealed no signs of pesticide contamination. Forty-four samples (20.9%) of the analyzed samples contained pesticide residues above MRLs, whereas 145 samples (68.7%) had levels at or below MRLs. This illustrates the pervasiveness of pesticides and their persistence in certain foods.

These results can be attributable to pesticide application procedures and crop-specific variables. Interestingly, crop-specific sensitivity to diseases and pests has a crucial effect. For instance, significant pesticide residue percentages were found in vegetables such as ocimum, parsley, and grape leaves, presumably as a result of these plants' increased susceptibility to certain pests (Keikotlhaile and Spanoghe 2011; Asiri et al. 2020). The moderate residual percentages seen in Jew's Mallow, Mint, and Spinach, however, may be a sign of their moderate susceptibility. These changes are consistent with other studies by (Juraske et al. 2012; Fantke and Jolliet 2016), highlighting crop-specific traits' influence on pest control techniques and, subsequently, residue levels. These changes are consistent with the study by (Barzman et al. 2015), which highlights the influence of crop-specific traits on pest control techniques and, subsequently, residue levels. Disparities in pesticide application techniques, which include elements like dose and frequency of treatment, were revealed as major causes, suggesting the role of application strategies in determining residue levels in plants (Mengistie et al. 2017).

In addition, the detected pesticide residue percentages were significantly influenced by the features of the vegetable surface, the date of the harvest, and the environmental factors (Bajwa and Sandhu 2014; Chung 2018). Vegetable surface characteristics have been shown to have a considerable impact on residue retention (Chen et al. 2020; Umapathi et al. 2022). Celery, arugula, purslane, and lettuce had lower percentages, which may be explained by the fact that vegetables with rough surfaces may retain more residues (Shah et al. 2011). Furthermore, it is important to consider harvest timing and the management procedures after harvest. Vegetables harvested later or going through less rigorous washing and processing may retain higher residues (Bajwa and Sandhu 2014; VanWoerkom et al. 2022). It is well recognized that environmental conditions, notably regional climate and insect pressure, influence the need for pesticides (Miraglia et al. 2009; Meissle et al. 2010; Birch et al. 2011).

In turn, this study employed LC–MS/MS analysis in conjunction with a comprehensive reference standard database consisting of 237 entries to quantify the pesticide's concentration and diversity precisely. Interestingly, the analysis revealed the presence of only 17 distinct pesticide residues within the tested samples, and strikingly, 14 of these exceeded the MRLs. The fact that the examined samples contained just 17 different pesticide residues raises concerns regarding the underlying causes. The historical pesticide use patterns in the areas where these crops are grown offer one reason (Yadouleton et al. 2009). Besides, the regional agricultural practices could determine whether Triallate, Metalaxyl, and Pyridaben are regularly used pesticides in these locations (Baig et al. 2009). Additionally, Tudi et al. (2021) highlighted that certain pesticides' persistence and degradation rates may be important. Certain pesticides may persist longer in the environment or on crops, which might result in greater residual levels (Tudi et al. 2021). Understanding their ubiquity necessitates a closer look at these pesticides' half-lives and environmental destiny (Shen et al. 2022).

Furthermore, pesticide formulations and application practices merit scrutiny. The formulation of these pesticides may influence their persistence and residue levels (Navarro et al. 2007; Kalyabina et al. 2021). Differences in application methods, including timing, frequency, and dosage, may further contribute to variations in residue detection.

The extensive genotoxicity database includes in vitro and in vivo studies evaluating point mutations, chromosomal effects, and DNA damage in prokaryotic and eukaryotic test systems. The database provides evidence that pesticide exposure can cause genetic damage, leading to cancer and other health problems (Elgueta et al. 2017). It can be concluded that farmers are not taking adequate precautions to apply insecticides at appropriate doses and standard pre-harvest intervals (Latif et al. 2011; Benaboud et al. 2021).

Overall, it is imperative to establish comprehensive policies that evaluate regulatory compliance and environmental factors, minimize pesticide contamination, ensure food safety, and protect public health. Adopting effective agricultural practices, including integrated pest management (IPM), crop rotation, and biological control techniques, is crucial to reducing the danger posed by pesticide residues in food items. Additionally, consumers may lessen their exposure to pesticides by carefully washing their fruits and vegetables before eating them and, where feasible, buying organic products.

Conclusions

In conclusion, 60% of the examined leafy vegetables were free from pesticide residues. In comparison, 40% exhibited varying levels of pesticide contamination, with 18 samples falling within or below the (MRLs) and 22 samples surpassing these prescribed limits. Triallate herbicide emerged as the most frequently detected residue, suggesting the need for comprehensive scientific assessment and enhanced management of pesticide application practices. Establishing routine monitoring programs can be crucial in mitigating contamination risks and protecting public health. Furthermore, the effective collaboration between the Ministries of Environment, Water and Agriculture (MEWA) and the Saudi Food & Drug Authority (SFDA), along with improved farmer guidance and training, is essential to address this issue and mitigate the adverse effects of pesticide residues on the health of the Saudi Arabian population.

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Authors contributions Conceptualization, M.E, R.S.; Methodology, O.A, F.K.; Software, F.K.; Performed the experiment, R.S, M.M.; Data analysis, R.S.; Writing—original draft preparation, R.S.; Writing—review and editing, M.E.; Conducting a research and investigation process, O.A, A.M.; Supervision, M.E, A.M. All authors have read and agreed to the published version of the manuscript.

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Data availability Data will be available upon request from the corresponding author.

Declarations

Ethical approval This work didn't involve animals or human participants.

Consent to participate Not applicable.

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