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Diagnosis and Estimation of Pesticide Residues in The Five Most Common Leafy Vegetables in Basrah Province

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Abstract

This study investigated pesticide residues in some species of leafy vegetables that are most consumed throughout the year, which included Celery, Basil, Cress, Mint, and Purslane. It was collected from September 2019 to August 2020 monthly from a vegetable grocer in the main markets in Basrah province. The results showed that 40% of the samples were contaminated with residues of nine types of pesticides with high concentrations, and only one sample exceeded the MRLs. Cress was the most polluted crop with a percentage of 60%, followed by Purslane (50%), Celery (42%), and finally Basil and Mint at 30% and 25%, respectively. Leafy vegetables collected in the autumn season contained the most significant number of pesticide residues, while leafy vegetables collected in the summer contained the fewest.

Keywords: Pesticide, vegetables, contamination, Basrah

Introduction

Iraqi people usually utilize leafy vegetables in their daily food as a part of cultural heritage, especially the residents of the southern provinces, due to a significant amount of nutrients and minerals. In addition to being available locally, inexpensively, it does not require further preparation and cooking and is obtainable throughout the year. The leafy vegetable is defined as those herbal plants whose part or portions are eaten as support food or main dishes, and they may be aromatic, bitter, or tasteless. (Mensah *et al*., 2008). The nutrient content in different types of leafy vegetables varies considerably. However, it is added to the daily diet because it is known to be rich sources of vitamins, essential amino acids, antioxidants, and many minerals such as Fe, Ca, and P and contain a high percentage of fibers. (Gupta *et al*.,2005).

Leafy vegetables are affected by many plant diseases and are attacked by many pests during their short growth period; Therefore, farmers resort to spraying them with agricultural pesticides to preserve the crop and eliminate pests. However, at the same time, they can also turn out to be a source of toxic substances such as pesticides. (Sharma *et al*.,2010).

Contamination of Iraqi farming leafy vegetable products with pesticide residues had not yet been widely studied, and scientific information in this regard is rarely available. Here we present the first

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study in this field to diagnose pesticide residues in leafy vegetables grown on both banks of the Shatt al-Arab river and provide background information on these crops' residues' levels. “Quick, Easy, Cheap, Effective, Rugged and Safe” (QuEChERS) method for the multiclass, multi-residue analysis of pesticides in fruits and vegetables of pesticides using GC-MS detection was employed. The aim was to investigate the presence of pesticide residues in five species of leafy vegetables traded in local markets in Basrah province. Evaluate their safety for human consumption by comparing the concentrations calculated therein with Maximum Residue Limits (MRLs).

The term pesticide residues refer to pesticides or pesticide oxidation or degradation products that may remain in food grains, vegetables, and fruits after application to crops. Many of these chemical wastes, especially the chlorinated pesticide derivatives, have a strong tendency to accumulate at high levels in the bodies of living organisms and the environment. (Sachs *et al*., 2010).

2. Materials and methods

Basrah Province is the third-largest Iraqi province in terms of population, with a total population of about (three million) and the sixth-largest in Iraq in terms of area, occupying almost 19,070 km^2. (Al-Mayah *et al*., 2016). It overlooks the Arabian Gulf's head in its southeastern part, and the province borders extend between the two latitudes (31º 20'- 29 º 05' N) and a long arc (48º 20'- 46º 40' E). (Mohamed and Mushkel, 2017; Yassin and Al-Saad, 2019). Many crops are planted in it throughout the year during the two seasons of summer and winter cultivation. The estimated agricultural area planted with leafy vegetables in Basrah province all over a year, on both riverbanks of Shatt al-Arab river and watered from it and its branches for the summer season is lesser than the winter season which, distributed as shown in Table 1.

2.1 Samples collection

A total of 48 fresh samples of Celery (*Apium graveolens* L.), Basil (*Ocimum basilicum* L.), Cress (*Lepidium sativum* L.), Mint (*Mentha spicata* L.), and Purslane (*Portulaca oleracea* L.) were collected from September 2019 to August 2020 from Basrah province markets as indicated in Fig.1 and Table 2. Each commodity's sample weight was 1kg and was collected from the upper, middle, and lower shelves to give a similar sample. Purchased samples were placed in a sterilized polyethylene bags and transported directly to the laboratory for extraction process within 24 hours.

2.2 Methods

According to the Original version QuEChERS method, preparation was carried out as described in (Anastassiades *et al*., 2003). Each leafy vegetable (1Kg) was washed with distilled water, dried, chopped into fine pieces, well-milled in an electrical blender. Ten grams of the comminuted and homogenous sample were weighted on Aluminum foils and dried with oven until the mass change is constant to estimate water content in it. Another (10 g) of the same milled sample was weighed into a 50 mL Teflon centrifuge tube, 10 mL Acetonitrile was added by using solvent-dispenser, and a mixture of (4 g Magnesium sulphate anhydrous coarsely grained,1 g Sodium chloride, 1 g Trisodium citrate dihydrate and 0.5 g Disodium hydrogen citrate sesquihydrate) were added to previous Teflon centrifuge tube then centrifuged for 5 min.

Two ml of the supernatant was transferred to clean 10 mL single-use centrifuge tubes with screw caps which contain a mixture of 50 mg Primary-Secondary Amine, 300 mg magnesium sulfate anhydrous fine powder, and 15 mg Graphitized Carbon Black. The tubes were shaken vigorously and centrifuged for 5 min. After that, 1 mL of cleaned extract was transferred into a 1.5 mL vial, and pH was quickly adjusted to 5 by adding a 10 µL. of

5 % formic acid solution in acetonitrile (vol./vol.). The pH-adjusted extract filled into vials became ready for further analysis by Gas Chromatography-Mass Spectrometry (GC-MS).

2.3 Gas chromatography-mass spectrometry (GC–MS)

Gas chromatography-mass spectrometry (Model Agilent 7693) with Gas chromatography (7890 B) and Mass spectrometry (Agilent 5977 A) detector equipped with a Splitless injector. HP-5MS capillary column (5% phenyl methyl siloxane: 30m × 0.25 mm × 0.25µm) was used in combination with the following oven temperature program: initial temperature 40 ^{O}C hold for 5 min, Ramp to 280 ^{O}C at 10 ^{O}C/min, Final temp. 310 ^{O}C held to end-run.

Table 1. Agricultural regions dedicated to growing leafy vegetables and their regional distribution in Basrah Province.

Region / Season	Agricultural regions and Area in Acres [a]												total
	Qurna	Midaina	Deir	Haretha	Iz Aldein saleem	Shatt Al-Arab	Nashwa	Imam Sadeq (A)	Seebah	Abu Alkhaseeb	Fao	Imam Qaem (Aj)	12
Summer 2019	80	100	450	50	150	300	50	400	10	150	100	50	1890
Winter 2020	32	100	450	100	150	480	50	400	13	140	150	250	2315
Summer 2020	125	100	450	60	60	400	50	425	10	100	100	50	1930

[a]ccording to the agricultural plan of the (Basrah Province Agriculture Directorate,2020).

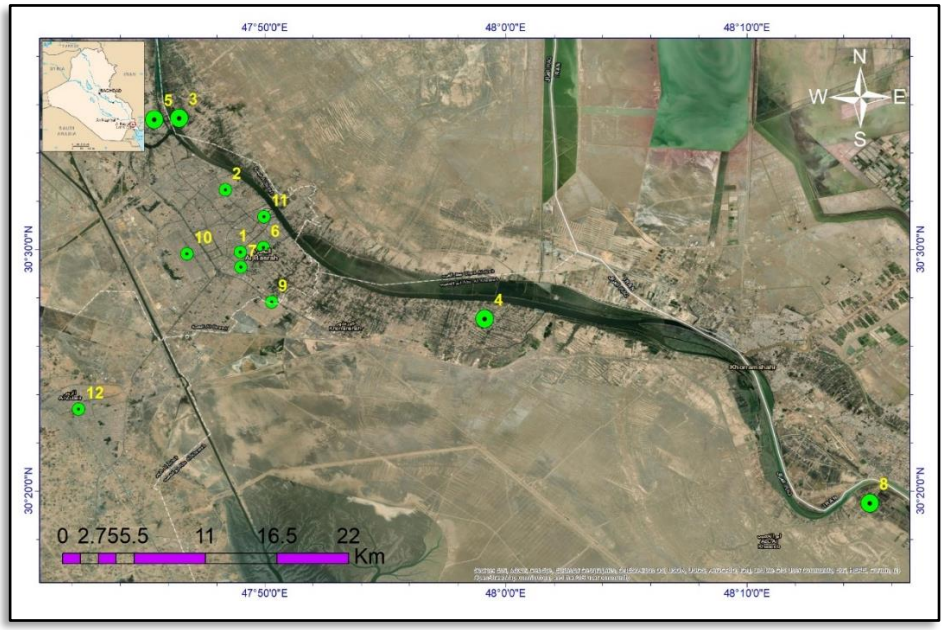

Fig.1. Map showing sample collection sites.

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Table 2. Sources, Date, Types, and Sum of collected Samples.

No.	Source	Date	No. of Sample types.				
			Celery	Basil	Cress	Mint	Purslane
1	Basrah market	Sep.2019	1	1	0	1	1
2	City center	Oct.2019	1	1	1	1	1
3	Haretha	Nov.2019	1	1	1	1	0
4	Abu Alkhaseeb	Dec.2019	1	0	1	1	0
5	Qarmat Ali	Jan. 2020	1	0	1	1	0
6	Shanshal mall	Feb.2020	1	1	1	1	0
7	Basrah center3	Mar.2020	1	1	1	1	1
8	Seebah	Apr.2020	1	1	0	1	1
9	Basrah's main Alwa	May.2020	1	1	0	1	1
10	Al Qisem	Jun. 2020	1	1	0	1	1
11	Al Ashar	Jul.2020	1	1	0	1	1
12	Al Zubair	Aug.2020	1	1	0	1	1
Total	12 markets	12 months	12	10	6	12	8
			48				

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3. Results and discussion

The detection of pesticides in the most consumed leafy vegetables throughout the year from several markets in Basrah province (south of Iraq) was the main aim of this study. Therefore, detecting pesticide levels seems to be an important contemporary public health problem to guarantee food quality and evaluate nutritional risk.

3.1 water content in samples

As shown in Fig. 2, the water content (moisture) in all samples of leafy vegetables selected for the study exceeded 80%. These results allow the QuEChERS method to be applied directly to selected leafy vegetables without raising the moisture content by adding distilled water. This result is consistent with the results of other studies such as Santos and Silva (2008), Vieira Potter (2011), who described fruits and vegetables as having higher water content may exceed 90% as well as Casanova (1996).

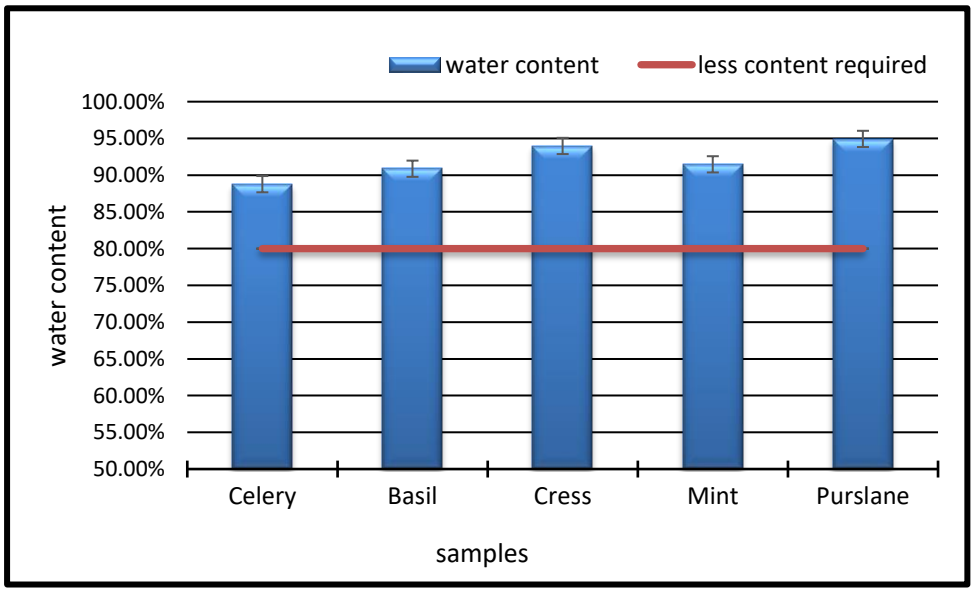

Fig. 2. Water content (moisture) in selected leafy vegetables.

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3.2 The diagnosed pesticide residues.

Twenty-four pesticide residues belonged to 9 types of pesticide with different chemical groups were found in 19 samples. All residues were diagnosed using a GC-MS device. Unfortunately, some samples contained more than one residue. Pesticide residues were not detected in 29 samples (60%). In comparison, 19 samples (40%) have. One contaminated sample was above the MRLs established by the Codex Alimentarius Commission. However, 18 samples (37.5%) contained pesticide residues without established MRLs, as shown in Table 3.

Table 3 Shows (MRLs) of the detected pesticides in tested leafy vegetables. No MRLs are yet established for all diagnosed pesticides except Cypermethrin. When pesticide residues exceed the MRLs established specifically for a specific pesticide on a particular foodstuff, they will constitute a violation of lawful limits. The widespread form of violated residues occurs when detected on a foodstuff for which tolerance has not been established. (Katz & Winter, 2009; Osman, *et al*., 2011).

We found the most frequent pesticide in all samples with Seven replicates, and the highest concentration (3.518 mg/kg) in Cress, Celery (1.932 mg/kg) was the insecticide Chlorpyrifos. Oleic acid with Five replicates and the higher concentration values of (0.406 mg/kg) in Purslane. Naphthalene and Metalaxyl were found out with three replicates in Four leafy vegetables, respectively. The results also showed that Cypermethrin was found in Basil and Mint, exceeding Basil's MRLs. In contrast, Benzoic acid, Cyhalothrin, Azoxystrobin, and Difenoconazole were found only in Cress with only One replicate.

Table 3. Maximum residue levels of the diagnosed pesticides.

Pesticides	Maximum residue levels, MRLs (mg/kg) an in tested leafy vegetables				
	Celery	Basil	Cress	Mint	Purslane
Benzoic acid	NS	NS	NS	NS	NS
Oleic acid	NS	NS	NS	NS	NS
Naphthalene	NS	NS	NS	NS	NS
Chlorpyrifos	NS	NS	NS	NS	NS
Cyhalothrin	NS	0.7	NS	NS	NS
Azoxystrobin	5	NS	NS	NS	NS
Difenoconazole	3	NS	NS	NS	NS
Cypermethrin	0.7	0.7	0.7	0.7	0.7
Metalaxyl	NS	NS	NS	NS	NS

According to the Codex Alimentarius Commission (FAO/WHO,2020).

[b] NS means MRL has not been established yet.

Eight types of pesticide residues were detected in Cress, ranging from (3.518 to 0.112 mg/kg). Four types of pesticide residues were detected in Mint and three in

Basil, while Tow types of pesticide residues were detected in Celery and Purslane, respectively. Unfortunately, we could not detect which one of these pesticide residue levels were exceeded the MRLs due to the few datasets of residue trials available, which do not include sufficient information to evaluate the suitability of the products for consumption or not as MacLachlan & Hamilton (2010) mentioned. Not setting (MRLs) for certain types of pesticides on some crops does not mean that they are safe and do not pose a threat to humans at any concentration or that they are exempt from the permissible limits.

Table 4 listed the details of 9 diagnosed pesticide residues that resulted in 19 violations and their registration status. The National Committee registers six types for Registration and Approval of Pesticides in the Iraqi Ministry of Agriculture, and three are not.

Table 4. The usage, chemical group, molecular weight, retention times, and Registration status in the Iraqi Ministry of Agriculture for diagnosed pesticides by GC–MS.

Pesticide	Use	Chemical group	Rt (min)	MW	Registration status
Benzoic acid	Insecticide/ Fungicide	Acids	12.928	122.12	NR
Oleic acid	Insecticide	Acids	21.499	282.5	NR
Naphthalene	Insecticide	Hydrocarbon	11.566	128.17	NR
Chlorpyrifos	Insecticide	Organophosphate	21.310	350.6	R
Cyhalothrin	Insecticide/ Fungicide	Pyrethroid	26.469	449.8	R
Azoxystrobin	Fungicide	Strobilurin	30.732	403.4	R
Difenoconazole	Fungicide	Triazole	29.809	406.3	R
Cypermethrin	Insecticide	Pyrethroid	28.218	416.3	R
Metalaxyl	Fungicide	Phenyl amide	20.583	279.33	R

R: registered; NR: not registered.

Four insecticides group (Oleic acid, Naphthalene, Chlorpyrifos, and Cypermethrin) were responsible for 17 samples (71%) of these violations. The highest concentration of insecticide residues was (3.518 mg/kg) by Chlorpyrifos, followed by Cypermethrin (1.477 mg/kg), Oleic acid (0.406 mg/kg) and also Naphthalene (0.544 mg/kg). In the fungicides group, three types were responsible for five samples (21%) of violations. The highest concentration was obtained for Metalaxyl (0.538 mg/kg), followed by Difenoconazole (0.372 mg/kg) and Azoxystrobin (0.266 mg/kg), finally, in case of insecticide/ fungicide which were responsible for 2 samples (8%) only of these violations. These residues ranged from (1.738 mg/kg) for Benzoic acid to (0.147 mg/kg) for Cyhalothrin. Table 5 illustrates the levels and occurrence of pesticide residues in tested leafy vegetable samples.

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Table 5. Levels (mg/kg) of pesticide residues in leafy vegetables are collected from several Basrah governorate markets.

Sample	Date	Season	Pesticides	Pesticide level, mg/kg
Celery	Sep.2019	Autumn	Chlorpyrifos	1.932
	Oct.2019	Autumn	Chlorpyrifos	0.591
	Nov.2019	Autumn	Naphthalene	0.528
	Dec.2019	Winter	Naphthalene	0.544
	Apr.2020	Spring	Chlorpyrifos	1.836
Basil	Mar.2020	Spring	Metalaxyl	0.538
	Apr.2020	Spring	Chlorpyrifos	0.699
	Jun.2020	Summer	Cypermethrin	1.477[a]
Cress	Nov.2019	Autumn	Benzoic acid	1.738
			Naphthalene	0.123
	Dec.2019	Winter	Oleic acid	0.372
	Feb.2020	Winter	Metalaxyl	0.218
			Difenoconazole	0.344
			Azoxystrobin	0.266
	Mar.2020	Spring	Chlorpyrifos	**3.518**
			Cyhalothrin	0.147
Mint	Dec.2019	Winter	Chlorpyrifos	0.227
			Metalaxyl	0.133
	Jan.2020	Winter	Oleic acid	0.163
	Mar.2020	Spring	Cypermethrin	0.303
Purslane	Sept.2019	Autumn	Oleic acid	0.090
	Oct.2019	Autumn	Chlorpyrifos	0.112
	Jul.2020	Summer	Oleic acid	**0.017**
	Aug.2020	Summer	Oleic acid	0.406

[a] Exceed the MRL.

Fig. 3 shows that 48 samples consisting of five leafy vegetables were collected from several markets and analyzed to detect pesticide residues from different chemical groups, of which 19 samples contained pesticide residues. The statistical analysis results showed no significant differences at the probability level ($P \leq 0.05$) between samples in the number of detected pesticide residues.

The highest diversity of pesticides was recorded during December and March (3 types each), followed by September, October, November, and April (2 types each), January, February, June, July, and August. They contained only one pesticide type each. At the same time, May has not recorded any pesticide residues. Fig. 4 illustrates the distribution of pesticide residues in tested leafy vegetable samples

during the months of study. For summer planting, the effect of high temperatures helps accelerate the process of pesticide fading (Al-Mashhadany *et al*., 2012). This effect is evident in the spring months, with the onset of high temperatures, and during the summer, the number of residues decreased to 1. Generally, the statistical analysis results confirmed no significant differences at the probability level (P≤0.05) between the months of the study in the number and concentrations of pesticide residues.

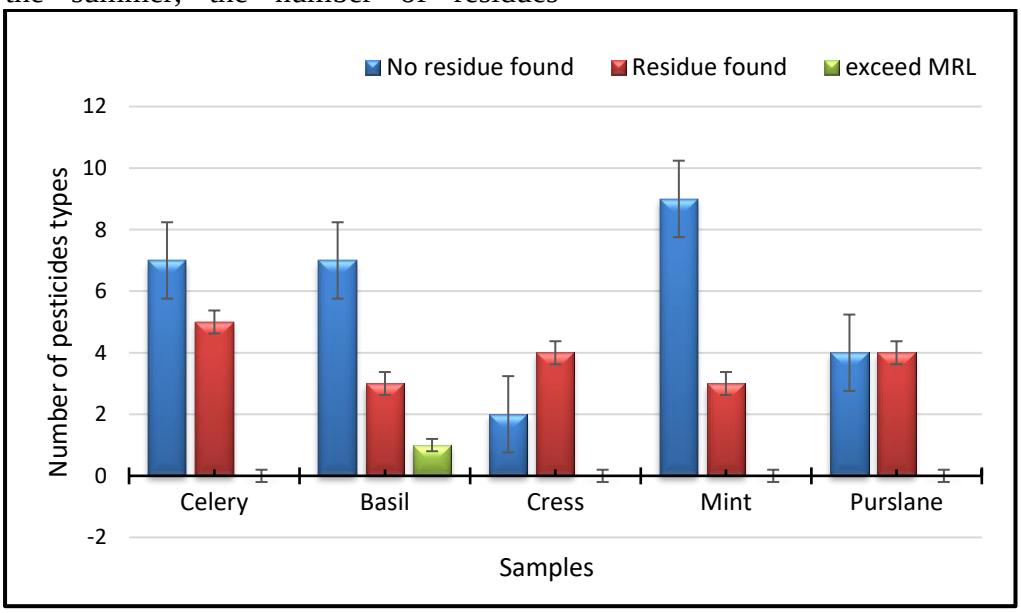

Fig. 3. The occurrence of pesticide residues in the leafy vegetables studied samples and their sources.

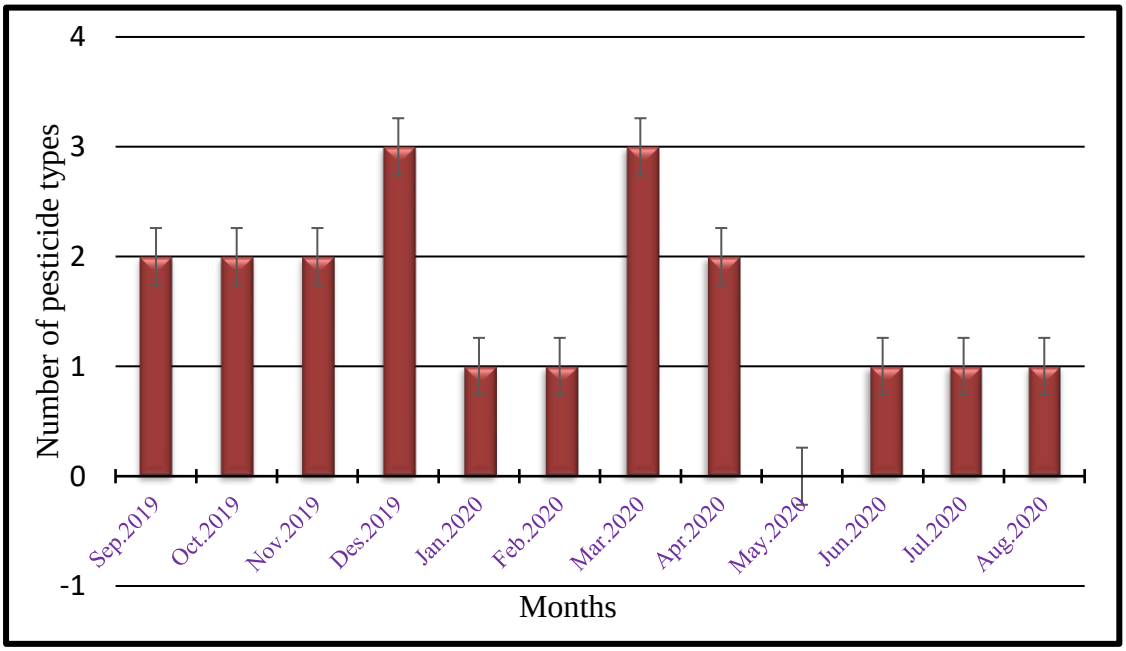

Fig. 4. Distribution of pesticide types in tested leafy vegetable samples during the year of study.

Leafy vegetables are susceptible to contamination with different pesticide residues in the field due to either direct application of pesticides, soilborne pesticides, or contaminated water for irrigation (Ingham *et al*., 2005). Besides marketing or storage quality. The reason for the presence of such a large number of pesticide residues in leafy vegetable crops is probably due to some bad agricultural practices by farmers; because these herbaceous crops during their short life period are characterized by density, rapid growth, and early maturity, accompanied by a small surface area of leaves and their low height above the soil surface, making them an easy target for many pests, which needs to be treated with agricultural pesticides and harvested in short periods that may not approach the recommended safety periods, thus reducing the rate of depletion of the pesticide residues applied to them; Added to this, another fact that it is usually consumed fresh directly after washing without undergoing any heat treatment, peeling, or even prolonged soaking in saline solutions. This conclusion is completely consistent with what Naddaf (2015) mentioned the method of preserving and consuming leafy vegetables "Food preservation by using cold, drying, acidity, dehydration, heating, chemical conservation, and irradiation works out with different fruits and vegetables but does not allow fresh leafy vegetables to be consumed as such." There is no method to store leafy vegetables without causing damage to the leaves and affecting the relish and shape. As a result, it required earnest precaution while the short handling process from harvesting until the consumption minute. It may also be planted periodically next to or under the shade of other crops, such as palm trees, lemons, and others, which leads to contamination with many types of pesticides designated for these crops or absorbed from the contaminated soil. The present study's conclusion is consistent with what Qin *et al*. (2015) concluded during their study of various leafy vegetables. The other reason may be due to the storage manner and unhealthy marketing method used by greengrocers to preserve its glossy and freshness. They wrap it with burlap bags, which may be unclean and re-used. They continue to soak or moisten them with water that is likely to be polluted, chlorinated in unconscious quantities, or naphthalene balls or other types of other sterilizers that have been popular in recent times.

4. Conclusions

The contamination shown in our results indicates the possibility that the consumption of leafy vegetables containing pesticide residues may be the cause of outbreaks of some diseases and poisoning cases. The results also showed: Chlorpyrifos and Oleic acid are the most common pesticide in leafy vegetables, Cress and Purslane are the most polluted leafy vegetables with pesticide residues, farmers mix and spray more than one pesticide and do not adhere to the proven dilution ratios and safety period, irrigation water is a significant source for transporting pesticide residues to humans via leafy vegetables, the autumn crops were the highest in several residues, while the summer crops were the lowest.

Therefore, we urge the application of international standards to ensure the quality of agriculture in the present and the future and preserve the consumer's health and commitment to applying green pesticides intended for leafy vegetables, which are characterized by little toxicity and Its safety period is relatively short.

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تشخيص وتقدير بقايا المبيدات الحشرية في خمس من الخضراوات الورقية الأكثر شيوعاً في محافظة البصرة

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المستخلص

تتحرى الدراسة الحالية عن تواجد متبقيات المبيدات في بعض انواع الخضروات الورقية الاكثر استهلاكاً على مدار السنة وهي الكرفس، الريحان، الرشاد، النعناع والبربين والتي جمعت للفترة من ايلول 2019 ولغاية آب 2020 بشكل شهري من تجار الخضروات في الاسواق الرئيسية في المحافظة. أظهرت النتائج احتواء (40%) من العينات بمتبقيات لـ تسعة انواع من مبيدات الافات وبتراكيز عالية، وان عينة واحدة فقط قد تجاوزت الـ (MRLs). وجد ان الرشاد أكثر المحاصيل تلوثاً بنسبة (60%)، يليه البربين (50%)، الكرفس (42%) واخيراً الريحان والنعناع بنسب (30%) و(25%) على التوالي. احتوت الخضروات الورقية التي تم جمعها في موسم الخريف على أكبر عدد من متبقيات المبيدات ، بينما احتوت الخضروات الورقية التي تم جمعها في الصيف على أقل عدد.