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(Original Article)

Monitoring of Some Pesticide Residues in Grape Samples in Assiut Governorate, Egypt

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Abstract

In Egypt, grapes, *Vitis vinifera* are an important agricultural export crop. The use of various synthetic pesticides is essential to control diseases and pests for export purposes. Pesticide residues are a significant concern for food safety and quality in the Egyptian grape industry. This study evaluated the concentrations of pesticide residues in grape samples taken from five local markets in Assiut Governorate using LC-MS/MS. Fourteen types of pesticide residues were identified. The data from this study indicated that some pesticide residues exceeded the maximum residue limit set by regulatory authorities. These pesticide residues included carbendazim, buprofezin, pendimethalin, metolachlor, thiamethoxam, clothianidin, dimethoate, and omethoate. Continuous research and policy actions are necessary to ensure the safety of the food supply. The study emphasized the need for improved pesticide management, oversight, and farmer education to ensure food safety and reduce the risk of harmful pesticide residues being consumed through grape consumption. Suggestions were made to strengthen legal frameworks and promote integrated pest management strategies in the grape industry.

Keywords: *Grapes, Monitoring, Pesticide residue, Environmental toxicology.*

Introduction

The cultivation of grapes is widely spread around the world with an estimated surface area of 7.6 million hectares in 2014 (Grimalt and Dehouck 2016). Grape production is an important activity due to the high nutritional properties of grapes and their ancient domestication leading to a large variety of by-products (Grimalt and Dehouck 2016). Although the use of pesticides in grapevine production provides various benefits, the presence of pesticide residues in grapes raises health concerns (Schusterova *et al*., 2021). Pesticide residue in grapes has increasingly aroused the attention of consumers. Certain intakes of pesticide residue content may harm consumers' health (Ye *et al*., 2022). Pesticide residue monitoring is an obligation for making decisions on whether the utilization of certain pesticides is

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safe for consumers or not (Mahdavi *et al*., 2022). Many pesticides and insecticides are used to combat unwanted pests of grapes. Sometimes, pesticides are misused in grape cultivation, thus exceeding the allowable level of pesticide residues. Pesticide residues in grapes can damage the environment, affect the quality of grapes and their processed products, and concomitantly affect human health (Syrgabek and Alimzhanova 2022). Appropriate application of pesticides is not always carried out and even when good agricultural practices are performed, pesticides can accumulate during the growing stage of the plant or from post-harvest treatment (Nieto *et al*., 2015). Chemical pesticides including fungicides, insecticides, and herbicides, are increasingly used in grapes. Thus, pesticide residues are always detected in grapes, grape juices wines and consequently the product quality of wine will be affected. It is necessary to limit the quantity of pesticides (He *et al*., 2016). Therefore, the present study aimed to investigate the levels of pesticide residues in grape samples collected from five local markets in Assiut Governorate (Assiut City, Manfalut, Abo-Tig, Badari and Dairut) using the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method and liquid chromatography-tandem mass spectrometry (LC-MS/MS) procedures, then compared the concentrations found with their maximum residue limits (MRLs) based on EU-MRL standards.

Materials and Methods

Sample collection

One kilogram of grape samples was collected from five local markets in Assiut Governorate in September 2021 (Table 1). They were collected in polyethylene bags labeled with the name of the center date, then placed in the freezer to soften them and make it easier to blend them in the blender.

Table 1. Random markets from which the grape samples were obtained in Assiut Governorate, Egypt.

No.	Market location
1	Assiut city
2	Badari
3	Dairut
4	Manfalut
5	Abo-Tig

Sample Preparation, extraction and clean up

Sample preparations were previously described by Ahmed *et al.* (2019). Samples were chopped and milled to homogeneity using a blender. Pesticides were extracted using the QuEChERS method. After chopping and milling 10 g of each sample was placed into a 50 ml polyethylene tube. Twenty ml of acetonitrile was added to each tube. The samples were well shaken using a vortex mixer for 2 min. Afterward, 6 g of anhydrous magnesium sulfate and 1.5 g of sodium chloride were added, then extracted by shaking vigorously on a vortex for 5 min and centrifuged for 5 min at 5,000 rpm. An aliquot of 4 ml was transferred from the supernatant to a new clean 15 ml centrifuge tube containing 100 mg primary secondary amine (PSA) and 600 mg anhydrous magnesium sulfate. The samples were again

vortexed for 3 min and then centrifuged for 5 min at 5,000 rpm. Then transfer the supernatant to a 2 ml vial. The extracts were ready for analysis by LC-MS/MS.

Instrument conditions and Chromatograms of standard pesticides

The LC-MS/MS instrument was used to analyze grape samples (Table 2). However, chromatograms of standard demonstrated in Figures 1 - 12.

Table 2. LC-MS/MS instrument conditions

Instrument:	LC-MS/MS Tandam mass Spectrometer, Thermo Scientific TM Dionex Ultimate TM 3000 RS UHPLC+ focused system coupled to a TSQ Altis triple quadrupole mass spectrometer (Thermo Fisher Scientific, Austin, TX, USA).
Column:	a Hypersil GoldTM C18 column (100 × 2.1 mm, 3 µm film thickness.
Mobile phase:	A: water, B: methanol Both mobile phases contain 5 mM ammonium format and 0.1% formic acid.
Flow rate:	0.3 ml/min.
Injection Volume:	5 µl
Tray Temp:	40 °C

The mobile phase gradient program was 0–12 min 100% A, 12–14 min 0% A, 14.1-20 min 100% A. Ion production in mass spectrometry was achieved applying a voltage in appositive (H-ESI+) mode. The positive ion spray voltage was 3800 V. The sheath and Aux gas were 40 and 10 Arb, respectively. The ion transfer tube and vaporizer temperatures were 325°C and 350°C, respectively.

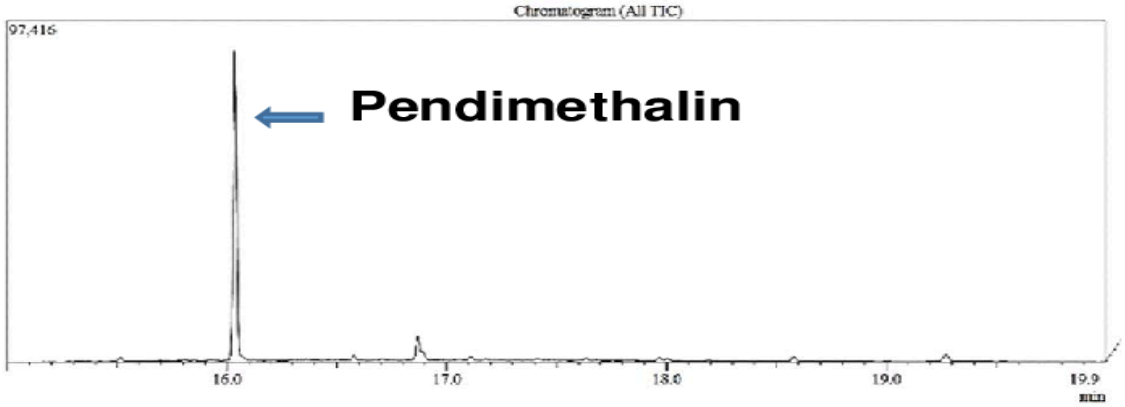

Fig. 1. Chromatogram of pendimethalin standard.

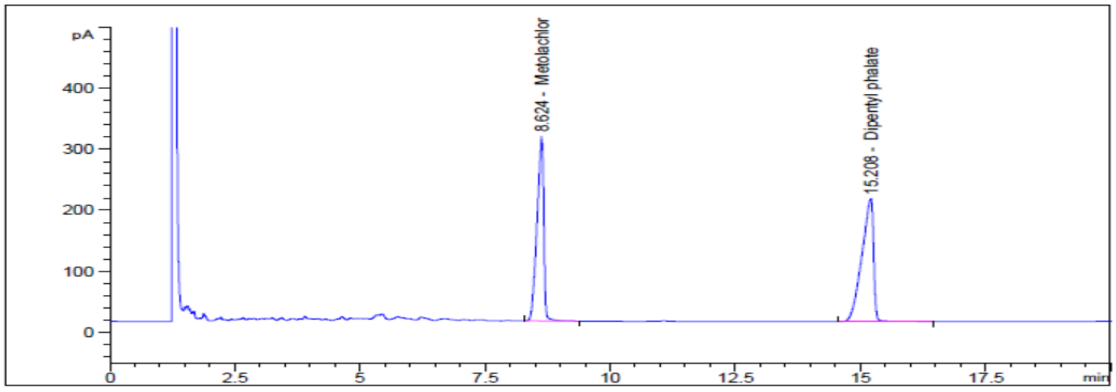

Fig. 2. Chromatogram of metolachlor standard.

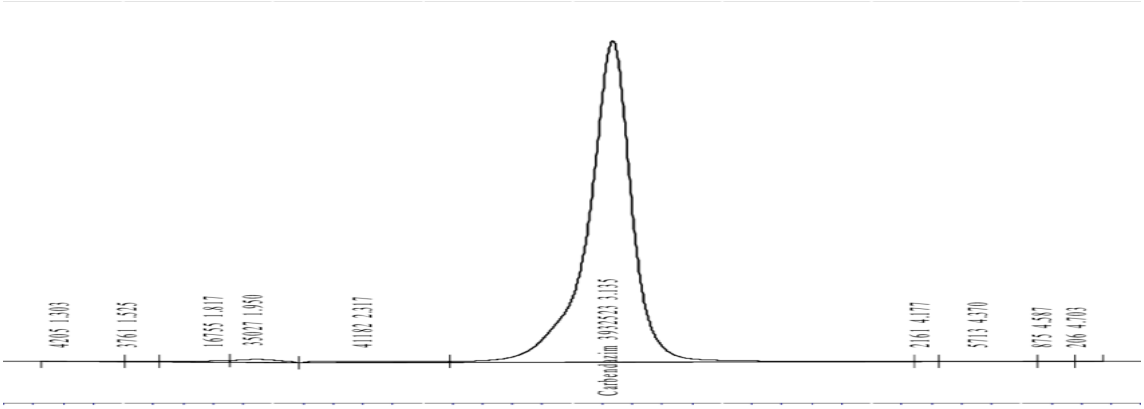

Fig. 3. Chromatogram of carbendazim standard.

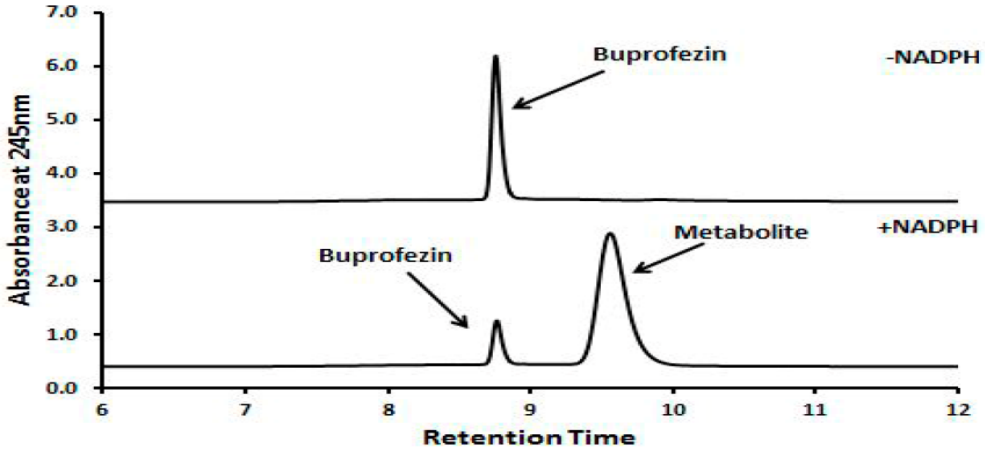

Fig. 4. Chromatogram of buprofezin standard.

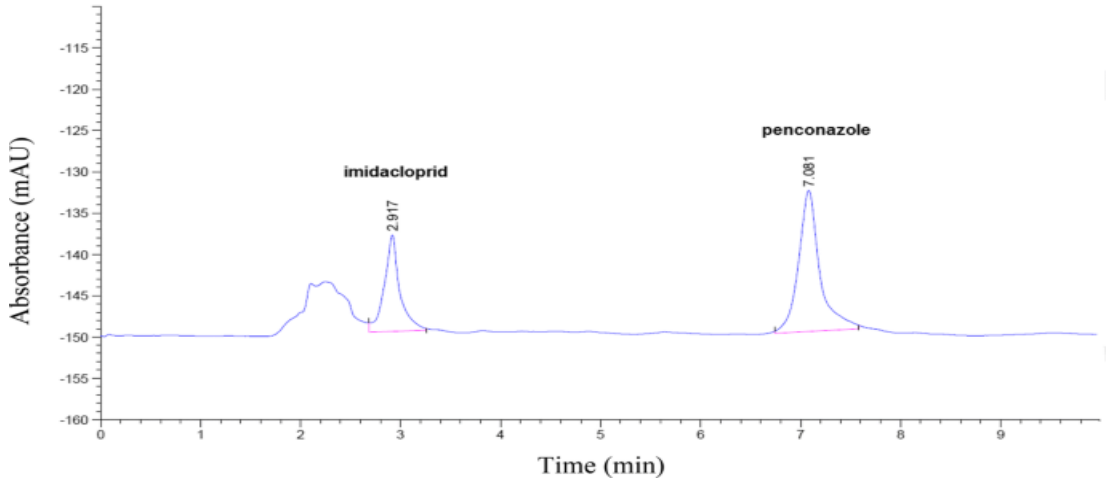

Fig. 5. Chromatogram of imidacloprid and penconazole standard.

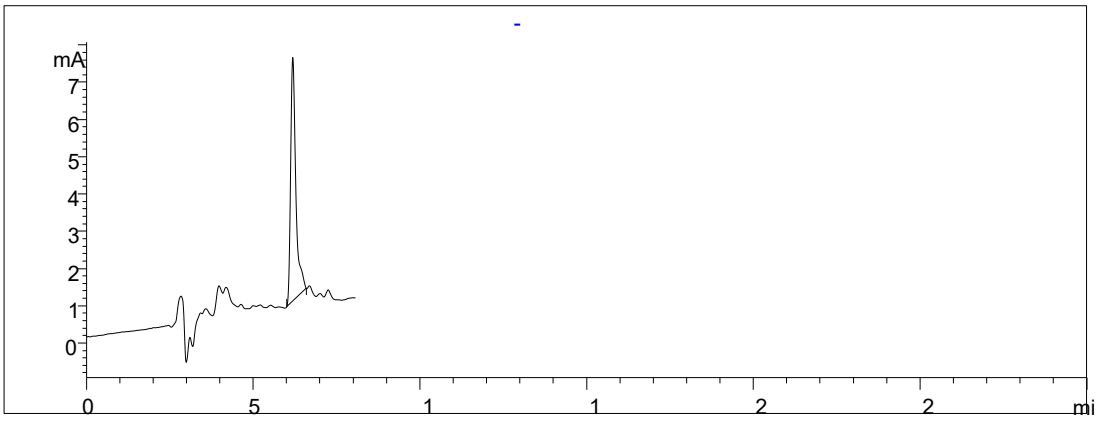

Fig. 6. Chromatogram of diclofop-methyl standard

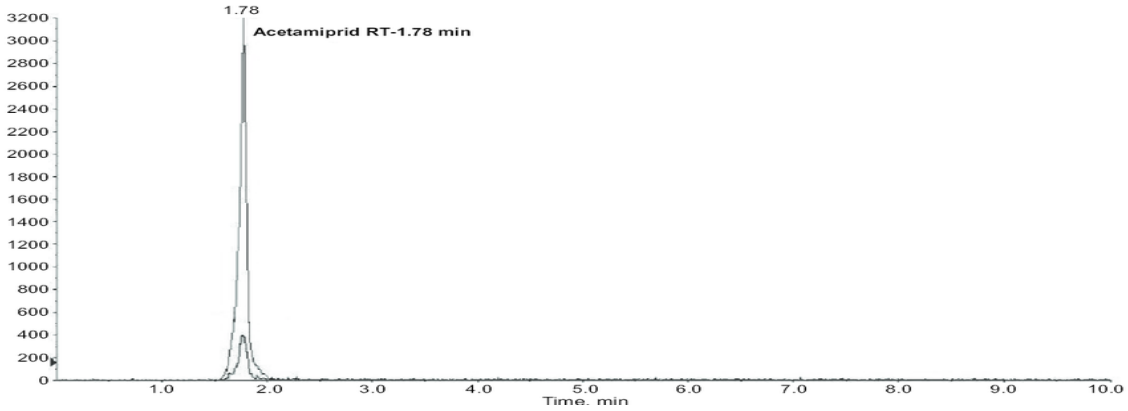

Fig. 7. Chromatogram of acetamiprid standard.

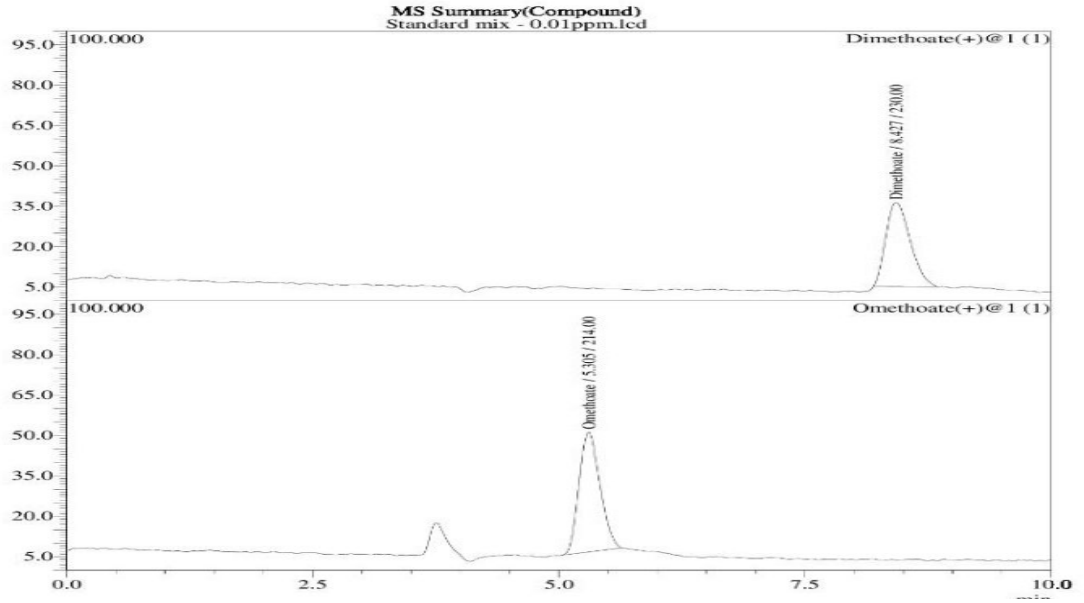

Fig. 8. Chromatogram of dimethoate and omethoate standard.

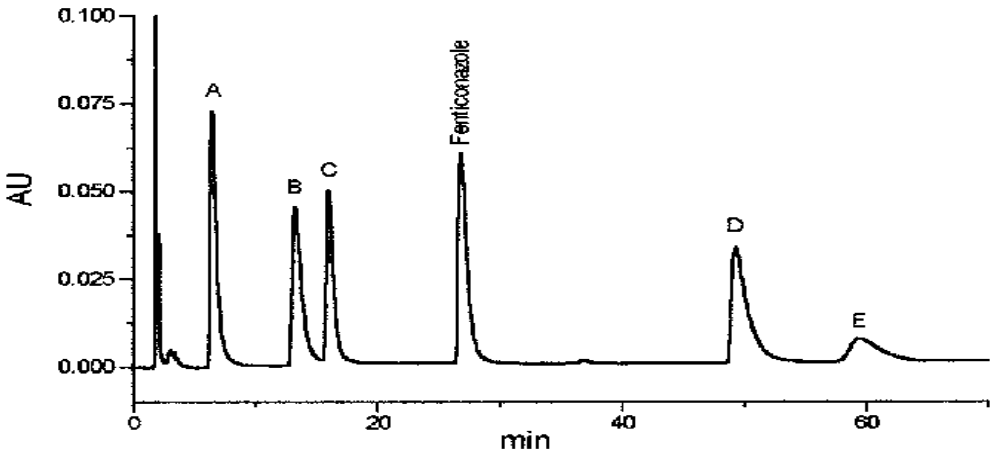

Fig. 9. Chromatogram of fenbuconazole standard.

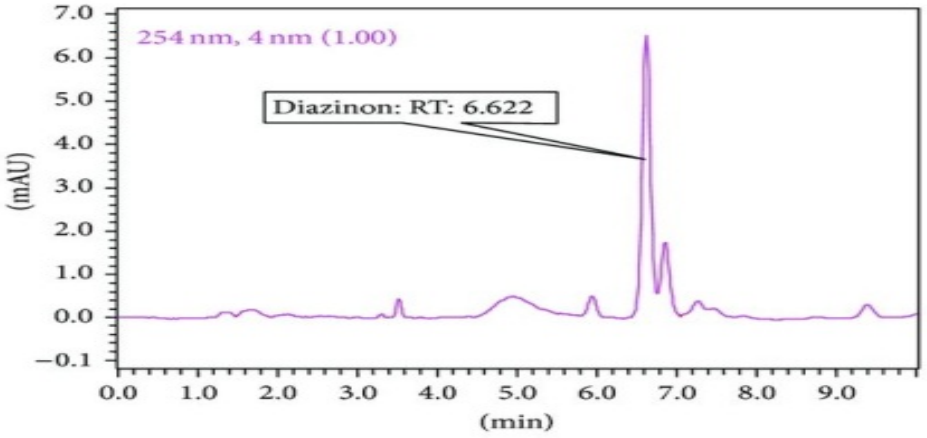

Fig. 10: Chromatogram of dizinon standard.

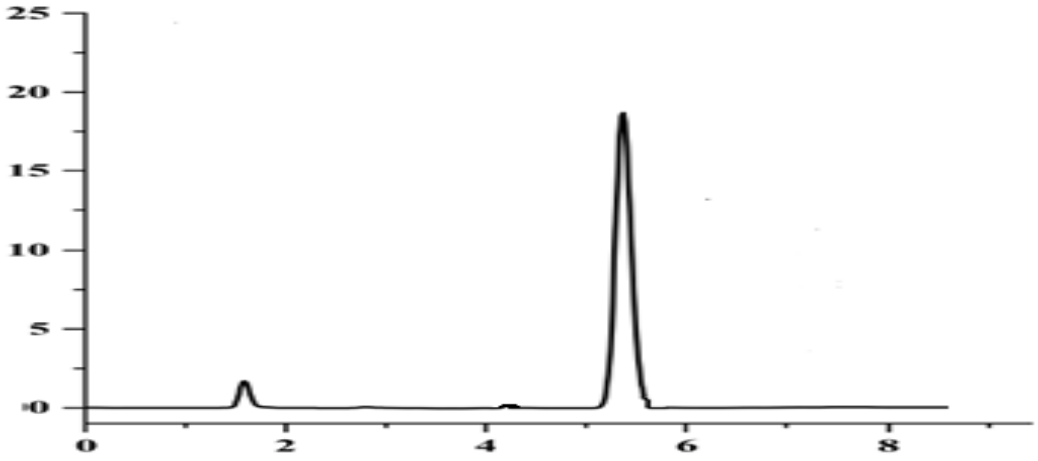

Fig. 11. Chromatogram of clothianidin and thiamethoxam standard.

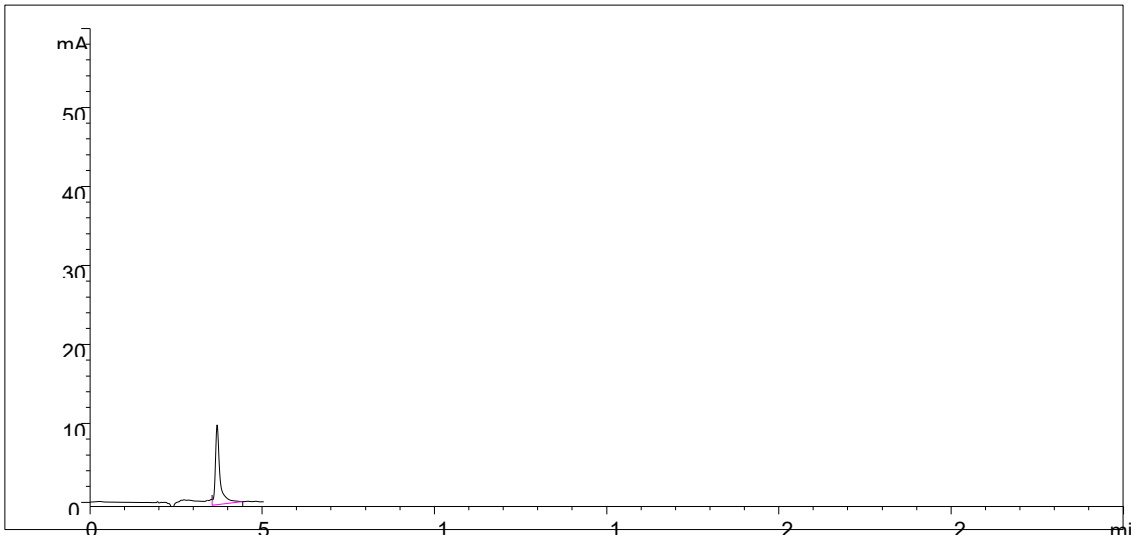

Fig. 12. Chromatogram of thiamethoxam standard

Method validation

Results were reported as ppm (mg/kg) in this study (Table 3). Replicate measurements of lowest concentrations spiked test samples at least 5 times. The lowest spike level (0.01 PPM (0.01 mg/kg) meets the method performance criteria for trueness (mean recoveries are within the range 84–104 % (acceptable range 70 – 120%) and precision (repeatability RSD ≤ 20 %). LOD ranged from 0.0003 to 0.0015 mg/kg and LOQ ranged from 0.001 to 0.005 mg/kg. It has been noted that LOQ < MRL. Precision (repeatability RSD ≤ 19 %) sample. Five levels of concentration are spaced across the linear range. Plot response (y-axis) against concentration (x-axis). The lower end of the working range is bounded by the limit of quantification LOQ. The results showed Linearity R^2 value ranged from 0.992 to 0.999 (acceptable range > 0.99).

Table 3. The average recovery percentage (spike level 0.01 ppm) and other validated parameters of analytes in grape samples.

No.	Analyte	Category	Average recovery %	SD	RSD%	LOD = 3*SD	LOQ = 10*SD
						mg/ kg	mg/ kg
1	**Imidacloprid**	Insecticide	95	0.0005	9	0.0015	0.005
2	**Pendimethalin**	Herbicide	88	0.0005	10	0.0015	0.005
3	**Carbendazim**	Fungicide	87	0.0005	14	0.0015	0.005
4	**Dimethoate**	Insecticide and Acaricide	92	0.0005	12	0.0015	0.005
5	**Omethoate**	Insecticide and Acaricide	96	0.0005	19	0.0015	0.005
6	**Buprofezin**	Insecticide	99	0.0001	16	0.0003	0.001
7	**Metolachlor**	Herbicide	84	0.0001	12	0.0003	0.001
8	**Clothianidin**	Insecticide	98	0.0005	8	0.0015	0.005
9	**Fenbuconazole**	Fungicide	94	0.0001	11	0.0003	0.001
10	**Penconazole**	Fungicide	99	0.0001	16	0.0003	0.001
11	**Thiamethoxam**	Insecticide	98	0.0005	15	0.0015	0.005
12	**Acetamiprid**	Insecticide	104	0.0005	15	0.0015	0.005
13	**Diazinon**	Insecticide	97	0.0001	11	0.0003	0.001
14	**Diclofop-methyl**	Herbicide	86	0.0001	6	0.0003	0.001

In Table 5 Levels of pesticide residues detected in grape samples were evaluated by comparing the national estimated daily intake (EDI) of Residue pesticide in grapes with the acceptable daily intake (ADI) as follows:

$$\mathbf{EDI = \Sigma\ C \times F / D \times W}$$

Where (EDI) is the daily intake estimate, according to national level (mg/kg, b.w.), C is the sum of the concentration of pesticide in each location (mg/kg), F is the mean annual intake of food per person, D is number of days in a year (365 days), and W is the mean body weight (assumed to be 80 kg). The annual intake per person of grapes in Egypt is 10.1 kg/person/year (CAPMAS, 2022). The health risk index (HRI) is considered the proportion of the estimated daily intake (EDI) to the accepted daily intake (ADI). ADI values were procured from the European Union Pesticides Database (2009). Health risk index (HRI) is calculated as: HRI = EDI / ADI.

Results and Discussions

In this study, we evaluated 15 grape samples and detected 14 pesticide residues in the tested grape samples. Data in Table (4) demonstrate the levels of pesticide residues in grape samples. The pesticide residues included 3 fungicides, 8 insecticides, and 3 herbicides.

The most frequent pesticide residues were carbendazim and clothianidin found in Badari, Manfalut, Abo-Tig, and Dairut markets, while dimethoate, and omethoate were found in Manfalut, Abo-Tig, and Badari markets. Pendimethalin, buprofezin and metolachlor were observed in Assiut city, Abo-Tig, and Badari markets. Thiamethoxam was found in Manfalut, Badari, and Dairut markets, and imidacloprid was observed in Assiut city, Abo-Tig, and Dairut markets. Penconzole was found in Assiut city, Badari and Dairut markets, diclofop-methyl was found in Assiut city and Badari markets, acetamiprid was observed in Manfalut and Badari markets and fenbuconazole was found in Abo-Tig and Badari markets. Furthermore, carbendazim exceeded the MRL by 0.213, 0.109, and 0.111 in Manfalut, Dairut, and Badari markets respectively. Buprofezin, pendimethalin and metolachlor exceeded the MRL by 0.050, 0.059, 0.114, 0.302, 0.011, and 0.113 respectively in Abo-Tig and Badari markets. Thiamethoxam exceeded MRL by 0.074, 0.076 and 0.006 respectively in Dairut, Abo-Tig, and Badari. Clothianidin exceeded MRL by 0.012 and 0.011 respectively in Dairut, Abo-Tig, and Badari markets. Dimethoate and omethoate exceeded MRL by 0.006, 0.037, 0.031, 0.063, 0.035, and 0.044 respectively in Manfalut, Badari, and Abo-Tig markets. Other pesticide residues were found within the MRL.

Table 4. Level of pesticide residues in grape samples from different markets in Assiut, Egypt

City	Market location	Pesticide	A.I. detected (mg/kg)	EU-MRL (mg/kg)	A.I. detected – MRL
Assiut Governorate	Assiut	Pendimethalin	0.032	0.05	< MRL
		Metolachlor	0.016	0.05	< MRL
		Imidacloprid	0.019	0.70	< MRL
		Buprofezin	0.006	0.01	< MRL
		Penconazole	0.001	0.50	< MRL
		Diclofop-methyl	0.001	0.02	< MRL
	Manfalut	Imidacloprid	0.045	0.70	< MRL
		Carbendazim	0.263	0.05	0.213
		Dimethoate	0.016	0.01	0.006
		Omethoate	0.047	0.01	0.037
		Acetamiprid	0.001	0.50	< MRL
		Clothianidin	0.005	0.01	< MRL
		Thiamethoxam	0.009	0.01	< MRL
	Dairut	Imidacloprid	0.365	0.70	< MRL
		Carbendazim	0.159	0.05	0.109
		Clothianidin	0.022	0.01	0.012
		Penconazole	0.002	0.50	< MRL
		Thiamethoxam	0.084	0.01	0.074
	Abo-Tig	Pendimethalin	0.164	0.05	0.114
		Metolachlor	0.061	0.05	0.011
		Carbendazim	0.003	0.05	< MRL
		Dimethoate	0.045	0.01	0.035
		Omethoate	0.054	0.01	0.044
		Fenbuconazole	0.044	1.50	< MRL
		Penconazole	0.020	0.50	< MRL
		Thiamethoxam	0.086	0.01	0.076
		Diazinon	0.001	0.01	< MRL
		Diclofop-methyl	0.003	0.02	< MRL
		Buprofezin	0.060	0.01	0.050
		Clothianidin	0.021	0.01	0.011
		Imidacloprid	0.094	0.70	< MRL
	Badari	Pendimethalin	0.352	0.05	0.302
		Metolachlor	0.163	0.05	0.113
		Carbendazim	0.161	0.05	0.111
		Dimethoate	0.041	0.01	0.031
		Omethoate	0.073	0.01	0.063
		Buprofezin	0.069	0.01	0.059
		Clothianidin	0.004	0.01	< MRL
		Fenbuconazole	0.005	1.50	< MRL
		Penconazole	0.016	0.50	< MRL
		Thiamethoxam	0.016	0.01	0.006
		Acetamiprid	0.050	0.50	< MRL
		Diazinon	0.004	0.01	< MRL
		Diclofop-methyl	0.010	0.02	< MRL

Data in Table 5 show the estimated daily intake values of pesticide residues and their corresponding health risk index in the grape samples. EDI stands for estimated daily intake and ADI represents the acceptable daily intake. Whereas an HRI value higher than 1 (≥100% of ADI) indicates an unacceptable chronic risk or is not safe for human consumption and is considered toxic. This study showed that the HRI value was less than one.

Table 5. Acceptable daily intake (ADI), estimated daily intake (EDI), and Health risk index (HRI) for pesticide residues found in grape samples

Pesticide	ADI	EDI	HRI (EDI/ADI)	Health risk
Pendimethalin	125	0.1895	0.0015	No
Metalachlor	30	0.0830	0.0028	No
Carbendazim	20	0.2148	0.0107	No
Buprofezin	10	0.0467	0.0047	No
Acetamipird	25	0.0176	0.0007	No
Penconzole	30	0.0135	0.0004	No
Diclofop-methyl	1	0.0048	0.0048	No
Imidacloprid	60	0.0547	0.0009	No
Thiamethoxam	26	0.0674	0.0026	No
Diazinon	0.2	0.0002	0.0012	No
Dimethoate	1	0.0353	0.0353	No
Omethoate	4	0.0636	0.0159	No
Clothianidim	97	0.0180	0.0002	No
Fenbuconzole	6	0.0159	0.0027	No

Grapes are a popular fruit that is treated with pesticides during cultivation to control pests, weeds and diseases that can impact crop yield and quality. Pesticide residues can remain on grape skin even after washing. Washing and peeling grapes can help reduce exposure, but some residues may still be present. There are growing concerns about the potential health and environmental impacts of pesticide use on grapes and other crops. Several studies dealing with the monitoring of pesticides in grapes have been published.

Hamzawy (2022) showed that the QuEChERS method, followed by GC-MS/MS and LC-MS/MS was used for determining more than 400 pesticide residues in grape leaves collected from the Egyptian markets for 2021 summer season. In his study, seventy-eight samples contained 36 pesticide residues of different chemical groups above the EU-MRLs. Nie *et al*., (2023) stated that the LODs ranged from 0.001 to 0.005 mg/kg, whereas the LOQs ranged from 0.003 to 0.015 mg/kg. These values are much lower than the MRLs set by China for grapes. The relative standard deviation was used to assess the accuracy of the analytical testing (RSD). The nine analytes had recoveries and RSDs in the concentration ranges of 85.4–93.8 percent and 8.2–15.8%, respectively. Mahdavi *et al*., (2022) recorded that residues of 85 pesticides in these products were investigated using modified QuEChERS extraction followed by UHPLC-MS/MS technique. Residues of 17 different pesticides were detected in some apple samples. In the grape sample, only 7 pesticides were detected. The levels of residues found in all apple and grape samples were below the maximum residue levels (MRLs) of Iran, except for iprodione. Health risk assessment associated with pesticide residues in apples and grapes was estimated by hazard quotient (HQ) and

hazard index (HI), which indicated that the HI value was lower than 1 in adults and children due to apple consumption. HI in adults and children were 0.012 and 0.054 in apples, and 0.001 and 0.003 in grape samples, respectively. Wang *et al*., (2018) found that the average recoveries of dimethomorph and pyraclostrobin in the grape and soil matrices varied from 76.88% to 97.05%, with relative standard deviations of 1.73%–10.38%. The degradation half-lives of dimethomorph and pyraclostrobin were 7.3–12.0 days and 3.6–7.0 days in grape and soil, respectively. The terminal residues of dimethomorph and pyraclostrobin in the two matrices were 0.05–0.87 mg/kg. Zhao *et al*., (2024) evaluated the average recoveries of pyraclostrobin ether ester, cyazofamid, and cyazofamid metabolite (CCIM) in grapes were 84–94%, 92–98%, and 99–104%, respectively. The relative standard deviations (RSDs) were 6.0–20.3%, 2.4–10.5%, and 1.3–4.0%, respectively, and the LOQs were all 0.05 mg/kg. The degradation dynamics of the experimental sites were in accordance with the first-order kinetic equation. The degradation half-lives of pyraclostrobin ether ester and cyazofamid were 17.8 d–28.9 d and 4.3 d–7.8 d, respectively. The final residues of pyraclostrobin ether ester and cyazofamid in grapes were <0.05 mg/kg.

In conclusion, pesticide residues were found in grape samples. Farmers often apply pesticides to fruits during the growing season to protect against pests, diseases, and weeds. Improper application methods, excessive use, or failure to follow recommended pre-harvest intervals can result in pesticide residues remaining on the fruit at harvest. Pesticides can contaminate soil, water, and air, leading to the uptake and accumulation of residues in fruits. Some pesticides are more persistent in the environment and can linger on or within fruits long after application. Farmers may use pesticides that are not approved for use on certain fruits or that have been banned, resulting in higher-than-expected pesticide residue levels. Some pesticides can accumulate in the tissues of fruits over time, leading to higher residue levels even with proper application. It's important for consumers to be aware of these potential causes and to buy organic produce or thoroughly wash conventional fruits to minimize exposure to pesticide residues. Regulatory agencies also play a key role in setting and enforcing residue limits to protect public health.

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رصد متبقيات بعض المبيدات في عينات العنب بمحافظة أسيوط بمصر

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الملخص

يعتبر العنب، *Vitis vinifera*، محصول تصديري زراعي مهم. في حين يعد استخدام المبيدات أمرًا ضروريًا لمكافحة الأمراض والآفات حتى تتمكن من زيادة الانتاجية والتصدير. ولكن تعتبر متبقيات المبيدات مصدر قلق رئيسي لسلامة الأغذية وجودتها في جودة محصول العنب في مصر. تم في هذه الدراسة تقييم تركيزات متبقيات المبيدات في عينات العنب المأخوذة من خمسة أسواق محلية بمحافظة أسيوط باستخدام تقنيةLC-MS/MS ، وتم اكتشاف 14 نوعاً مختلفا من متبقيات المبيدات. وتشير نتائج هذه الدراسة إلى أن بعض متبقيات المبيدات تجاوزت الحد الأقصى المسموح بها الذي حددته الجهات التنظيمية. وكانت متبقيات المبيدات هذه هي الكاربندازيم، والبيروفيزين، والبنديميثالين، والميتولاكلور، والثياميثوكسام، وكلوثياندين، والديميثوات، والأوميثوات. وهناك حاجة إلى إجراء أبحاث مستمرة وإجراءات رقابية لضمان سلامة الامن الغذائي. تؤكد الدراسة على الحاجة إلى إدارة أفضل للمبيدات، والرقابة، وتثقيف المزارعين من أجل ضمان سلامة الأغذية وتقليل مخاطر الاستهلاك لمتبقيات المبيدات الخطيرة من خلال تناول العنب. وتتناول هذه الدراسة مقترحات هامة لتشجيع وتعزيز وضع الأطر القانونية والتركيز على تطبيق الاستراتيجيات المتكاملة لمكافحة الآفات في محصول العنب.

الكلمات المفتاحية: *العنب، الرصد، متبقيات المبيدات، سمية البيئة*