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Assessing Pesticide Residue Levels in Fresh Grapes Grown in Siirt Province (Türkiye) and Selected Districts

Ruhan Ilknur Gazioglu Sensoy[1*], Firdevs Esen[2], Yagmur Yilmaz[3]

[1] Horticulture Department, Agricultural Faculty, Van Yuzuncu Yil University, 65090 Van, Türkiye
[2] TAGEM Directorate of Plant Protection Central Research İnstitute, Ankara, Türkiye
[3] The Institute of Natural and Applied Sciences, Van Yuzuncu Yil University, 65090 Van, Türkiye

* *Corresponding author: rigazioglu@yyu.edu.tr*

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ABSTRACT

This study aims to evaluate the residue levels of grape samples collected from vineyards in Siirt province and certain districts with intensive viticulture. Samples were obtained from 25 different vineyards in three districts of Siirt (Şirvan, Tillo, Merkez), and a screening was conducted for 255 pesticide active ingredients. The obtained results were compared with the pesticide concentration ranges determined by the Ministry of Agriculture and Forestry, published in the Official Gazette.

In the samples collected from Siirt city center, a total of 40 different pesticide active ingredients were detected in six samples, but the values were found to be significantly below the Maximum Residue Limit (MRL) range. In the samples from Tillo district, 30 different pesticide active ingredients below the MRL range were identified in four vineyards. As for the samples from Şirvan district, 86 different pesticide active ingredients were detected in 15 samples. Only one sample exceeded the acceptable MRL range (Malathion active ingredient), while 85 pesticides were found to be within the acceptable range.

The aim of this study was to shed light on residue issues in agricultural products and provide a foundation for future comprehensive studies. It attempts to present data that will contribute to a better understanding of residue problems and serve as a basis for more extensive research in the future.

Keywords: LC-MSMS, Grape, Pesticide, Residue.

1. INTRODUCTION

One of the most significant challenges resulting from the rapidly increasing world population is the food demand of consumers. According to the data from the Food and Agriculture Organization (FAO), approximately 40% of the current global population cannot meet their food requirements adequately, leading to thousands of deaths due to hunger and poverty every year. Despite the rapid population growth, the limited availability of agricultural land necessitates obtaining the highest possible yield per unit area. Today, it is estimated that without the use of agricultural pesticides, crop losses can reach around 65% on average for certain products. For instance, in wheat production, if pests such as weeds, rusts, bugs, and mites cannot be effectively controlled, the value of crop loss would be expressed in trillions. However, this loss can be minimized to a few billion units by investing in pesticide application (Karakaya et al., 1992).

Chemical substances used to combat living organisms that cause harm to plants and animals are collectively referred to as pesticides. Pesticides encompass hundreds of compounds with significantly different chemical structures and properties, such as insecticides (insect killers), fungicides (fungus killers), acaricides (mite killers), bactericides (bacteria killers), and others. There are approximately 1,000 companies worldwide engaged in the production of such pesticides, with about 90% of them focusing on the production of pesticide active ingredients. Most of these companies manufacture expired patents, while the number of firms involved in new synthesis studies is around 50. These companies conduct thousands of syntheses each year to develop new active ingredients. Currently, there are approximately 1,500 pesticide active substances available (Anonymous, 1980).

While the use of agricultural chemicals enhances agricultural production on one hand, their indiscriminate usage can directly or indirectly harm human health and ecosystems. When pesticides are applied above the recommended doses, excessive spraying is carried out, multiple pesticides are mixed and applied unnecessarily, or the required interval between the last application and harvest is not followed, they can leave excessive residues on food products. Consuming food with high levels of pesticide residues can lead to chronic or acute poisoning in humans and other organisms in the environment. Additionally, these pesticides can cause aroma and quality changes in certain products (Henning et al., 1954).

Approximately 3 million tons of pesticides are produced worldwide, with an approximate value of 30 million Euros (Durmusoglu et al., 2010). Pesticides exhibit high efficacy against harmful organisms, provide rapid results, and can protect crops from toxin-producing organisms. When used consciously and with control, they can yield positive economic outcomes (Delen et al., 2005). Pesticide usage varies among countries. For example, in our country, approximately 33,000 tons of pesticides are produced annually, with a value of 230-250 million dollars. In 2008, it was reported that 1,209 g of pesticide was consumed per hectare, totaling 20,032 tons of pesticide consumption (Delen et al., 2010). The distribution of pesticide consumption across regions revealed that the Marmara, Mediterranean, and Aegean regions accounted for 50% of the country's total consumption. The consumption in the Eastern and Southeastern Anatolia regions was approximately 10% (Durmusoglu et al., 2010).

As seen, pesticides are extensively used both globally and, in our country, holding significant economic importance. However, when applied to a plant, they do not remain solely on the surface or structure of that plant; they disperse into the environment through various means. Depending on their characteristics, pesticides can evaporate, be carried by wind, mix with rainwater and infiltrate groundwater or surface water, reaching far beyond the applied area. The dispersion and persistence of pesticides can vary based on light, water, environmental acidity, or soil properties, rapidly breaking down or, conversely, becoming more persistent.

In this study, table grape samples obtained from vineyards in Siirt province and its districts were evaluated for pesticide residues. The aim was to shed light on residue-related issues in agricultural products and present data that could contribute to more specific studies focusing on the pesticide usage levels in certain regions, which are planned to be conducted on a broader scale in the future.

2. MATERIAL and METHOD

2.1. Materials

The main material of the study consists of fresh grape samples collected from 25 different vineyards located in three different districts (Şirvan, Tillo, Merkez) of Siirt province.

2.2. Methods

Chemical Analysis The pesticide analysis was conducted using the AOAC 2007.01 method. A homogenized sample weighing 15 g was transferred into a 50 ml centrifuge tube. Then, 15 ml of acetonitrile solution containing 1% acetic acid was added and mixed. Next, Kit A (QuECHERS Extraction Salt 6 $MgSO_4$, 1.5 g NaOAc) was added to the same tube, and the cap was tightly closed. The tube was vigorously shaken for 1 minute and then centrifuged at 5000 rpm for 1 minute. From the upper phase, 6 ml was transferred to a 10 ml tube, and Kit B (QuECHERS cleanup kit 1200 mg $MgSO_4$ + 400 mg PSA) was carefully added. The tube was tightly closed, vigorously shaken for 30 seconds, and centrifuged at 5000 rpm for 1 minute. For LC-MS-MS analysis, 0.2 µm aliquot was taken into a vial after passing through a filter. To the vial, 0.5 ml of extract, 475 µl of 10.57 mM ammonium formate solution prepared in water, and 25 µl of acetonitrile were added. After closing the vial cap, it was gently shaken, placed in the autosampler, and injected. Readings were performed on an LC-MS-MS (Mass spectrometry-Mass Spectrophotometer) instrument (Anonymous, 2014).

The detected pesticide residue levels were interpreted according to the Turkish Food Codex, Pesticide Maximum Residue Limits Regulation Official Gazette 29099-24 August 2014 (Anonymous, 2014).

The residue levels obtained in the study were evaluated based on the "Turkish Food Codex (TFC) Communique on Maximum Residue Limits of Pesticides Allowed in Foodstuffs (Official Gazette: 21.01.2011-27822; Communique No: 2011/2)". The MRLs (Maximum Residue Limits) for each pesticide sample according to TFC are presented in Table 2.1.

Table 2.1. MRLs for Pesticides used in Fresh Grapes according to Turkish Food Codex

No		Pesticide Active Ingredient	Measurement Limit (µg/kg)	No	Pesticide Active Ingredient	Measurement Limit (µg/kg)
1		Acetamiprid	banned	17	Ethoprophos	20
2		Azoxystrobin	2000	18	fenhexamid	5000
3		Benalaxyl	300	19	Fenpyroximate	300
4		Benomyl	banned	20	Flusilazole	10
5		Bifenazate	700	21	Flutriafol	800
6		Bifenthrin	200	22	Formetanate	100
7		Boscalid	1000	23	Imazalil	20
8		Bromuconazole	500	24	Malathion	20
9		Bupirimate	1500	25	Metalaxyl	2000
10		Carbendazim	300	26	Myclobutanil	1000
11		Chlorpyrifos	500	27	Pyrimethanil	5000
12		Cymoxyanil	200	28	Quinoxyfen	1000
13		Cyprodinil	5000	29	Spirodiclofen	2000
14		Diethofencarb	10	30	Tebufenozide	3000
15		Difenoconazole	3000	31	Thifensulfuron-methyl	100
16		Dimethomorph	3000	32	Triflumizole	3000

3. RESULTS and DISCUSSION

Among the samples obtained from 25 vineyards examined in this study, one sample was found to have a residue value above the Maximum Residue Limit (MRL) range, while the rest of the samples were within the acceptable range.

In the samples taken from Siirt city center, 40 different pesticides were detected in 6 samples, but the values were found to be significantly below the MRL range (Table 3.1).

Table 3.1. Pesticide residue levels (µg/kg) in fresh grape samples from Siirt city center

ACTIVE SUBSTANCE	Bifenazate	Boscalid	Carbendazim	Chlorpyrifos	Cymoxyanil	Cyprodinil	Fenhexamid	Flutriafol	Formetanate	Malathion	Tebufenozide	Thifensulfuron-methyl
MRL Range	**700**	**1000**	**300**	**500**	**200**	**5000**	**5000**	**800**	**100**	**20**	**3000**	**100**
SAMPLES FROM SIIRT CITY CENTER	5.51	-	0.059	-	4.70	2.77	-	-	0.032	-	1.62	0.005
	-	-	-	0.043	4.83	3.08	-	6.31	0.065	6.49	1.82	0.004
	5.51	-	-	0.052	4.60	-	1.30	-	0.033	4.36	1.43	-
	5.51	-	-	0.037	4.63	3.24	-	6.48	0.030	-	1.32	0.004
	5.51	-	-	-	4.61	2.75	1.53	-	-	-	1.61	-
	5.52	0.083	-	-	4.62	-	1.46	6.49	-	-	1.39	-

In samples taken from Tillo district, 30 different pesticides were found below the MRL range, indicating permissible levels (Table 3.2).

Table 3.2. Pesticide residue levels (µg/kg) in fresh grape samples from Siirt/Tillo district

ACTIVE SUBSTANCE	Bifenazate	Boscalid	Carbendazim	Chlorpyrifos	Cymoxyanil	Cyprodinil	Fenhexamid	Flutriafol	Formetanate	Malathion	Tebufenozide	Thifensulfuron-Methyl
MRL Range	**700**	**1000**	**300**	**500**	**200**	**5000**	**5000**	**800**	**100**	**20**	**3000**	**100**
SAMPLES FROM TILLO DISTRICT	-	0.08	0.03	-	4.58	-	1.46	6.33	0.031		2.03	0.005
	-	-	-	0.042	4.50	2.48	1.19	6.64		8.81	1.58	
	-	-	-	-	4.62	-	-	6.85	0.031		1.61	0.004
	5.84	0.25	0.14		4.53	2.94	1.96	6.82		8.39	1.65	0.019

In samples obtained from vineyards in Şirvan district, 86 pesticides were detected in 15 samples. While one sample exceeded the MRL range for the active substance malathion (Figure 3.1), the residue levels in the other samples were found to be within the allowed range (Table 3.3).

Table 3.3. Pesticide residue levels (µg/kg) detected in fresh grape samples from Siirt/Şirvan district

ACTIVE SUBSTANCE	Bifenazate	Boscalid	Carbendazim	Chlorpyrifos	Cymoxyanil	Cyprodinil	Fenhexamid	Flutriafol	Formetanate	Malathion	Quinoxyfen	Tebufenozide	Thifensulfuron-Methyl
MRL Range	**700**	**1000**	**300**	**500**	**200**	**5000**	**5000**	**800**	**100**	**20**	**1000**	**3000**	**100**
SAMPLES FROM SİRVAN DISTRICT	-	-	-	-	4,55	-	1,30	-	0,03	11,00	-	1,69	-
	-	-	-	-	4,76	2,46	-	-	-	4,77	-	1,57	-
	-	-	0,09	0,02	4,65	-	-	-	0,07	-	-	1,78	0,01
	-	-	0,06	0,02	4,50	-	-	-	-	5,48	-	1,82	0,01
	5,52	-	0,10	-	4,63	-	-	-	0,01	-	0,05	-	-
	5,52	-	0,10	-	4,58	-	1,46	-	0,13	-	-	-	0,01
	5,84	-	-	-	4,66	2,90	1,30	-	0,01	5,31	-	2,58	0,01
	-	0,23	0,06	-	4,73	-	1,44	-	-	5,67	-		0,01
	-	-	-	0,04	4,69	2,25	2,09	-	0,03	9,65	-	1,67	-
	-	-	0,06	-	4,55			6,89	-	16,20	-	1,64	-
	6,46	0,63	-	-	4,77	2,47	1,19	6,74	0,03	**20,80**	-		0,003
	-	-	-	-	4,58	-	-	7,29	-	5,26	-	1,62	-
	-	-	-	-	-	2,46	-	6,48	-	7,34	-	1,40	-
	-	-	-	0,02	4,70	-	1,93	-	0,07	7,60	0,05	1,59	0,02
	-	-	-	-	-	-	-	6,57	-	-	-	1,48	0,01

The amounts of pesticide residues detected in fresh grape samples collected from different centers are given in the tables above. The MRL range indicates the maximum allowable residue limits for each active substance.

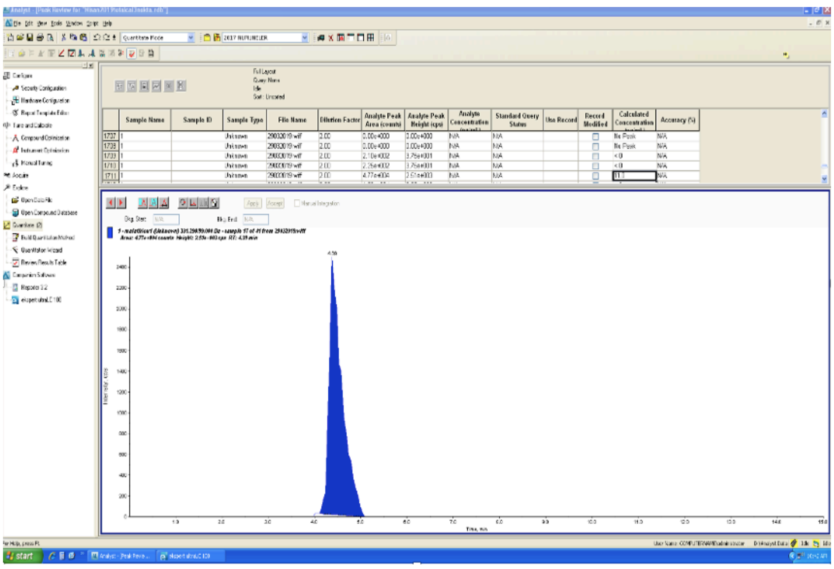

Figure 3.1. LC-MSMS image of Malathion active substance

In this study, a general pesticide screening was conducted on fresh grape samples obtained from 25 different vineyards, including the city center of Siirt and some districts. Screening was performed against 255 pesticide active substances, and 16 different pesticides were detected in the samples. In the majority of the samples, residue levels were found to be below the Maximum Residue Limit (MRL) values, which represent the highest allowable residue levels internationally. Except for one sample, residue levels were determined to be below the acceptable range (Table 3.1, 3.2, 3.3). The region's hot climate during the summer season has made it a vulnerable area in terms of diseases/pests, necessitating the use of pesticides or alternative control methods.

During the period of 2011-2015, pesticide residues were detected in 11.6% of the grape shipments exported from Alaşehir to the Russian Federation. MRL values for grapes vary between 0.05 and 50 mg/kg depending on the country, depending on the plant protection products. Biological-origin plant protection products do not have MRL values. Harvest intervals can also range from 0 to 90 days. Due to the varying residue limits and criteria for human health compliance depending on the target country's market, it is necessary to control all stages from production to export. Selecting and using plant protection products with different mode of action group codes to extend their efficacy capacity and prevent resistance formation will be crucial (Ozarik et al., 2018).

In a similar study, the aim was to determine the pesticide residue levels in fresh and dried grapes and pickled vine leaves from different regions such as Manisa, Kilis, Adıyaman-Besni, Siirt, Tokat, Balıkesir, Adana, and Mersin, which are considered as grape producers in our country, and even from countries with which we have border trade or import. The internationally recognized AOAC 2007.01 method, which is valid for pesticide analysis, was used, and screening for 250 different pesticides was conducted for each sample. Residues of 30 different pesticides were detected in all 16 samples of table grapes, including the presence of banned pesticides. In dried grapes, 10 different pesticides were identified; however, due to the lack of a national or international standard range value in this regard, the results could not be evaluated comparatively in terms of damage threshold values. For pickled vine leaves, different analyses and observations were conducted for both homemade pickles for personal consumption and commercially branded or unbranded pickled leaves. As a result, except for one sample available for sale as a commercial brand in supermarkets, no pesticide residues were found (Gazioğlu Şensoy et al., 2017).

The present study investigated the pesticide residue levels in early and mid-late season grape varieties obtained from two major supermarkets in Çanakkale province. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method, widely used for fresh vegetables and fruits, was employed for sample extraction and clean-up, while chromatographic separation was conducted using an LC/MS-MS instrument. The analysis revealed the presence of at least one pesticide residue in all ten samples of early season grape varieties. Different levels of Pyraclostrobin residue (ranging from 0.011 to 0.018 mg/kg) were detected in the samples. The Maximum Residue Limit (MRL) values for Pyraclostrobin in grapes are 1 mg/kg according to the Turkish Food Codex (TGK) and 2 mg/kg according to the FAO Codex. Thus, the detected residue levels were below the MRL values. The mid-late season grape varieties exhibited a relatively high number of pesticide residues. Boscalid residue was found in five samples; however, the detected residue levels for different pesticides were below the MRL values set by the EU and TGK (Dardeniz et al., 2018).

In a study examining the effects of various washing conditions on the removal of residues of three different pesticides (chlorpyrifos ethyl, acetamiprid, and penconazole) from grapes, it was observed that the residue levels of all three pesticides decreased with a decrease in washing water temperature. Comparing temperatures of 40 and 50 ˚C with 10, 20, and 30 ˚C, lower pesticide residue levels were obtained at the latter temperatures. Additionally, it was determined that a 4-minute immersion duration was not as effective as 2 or 3-minute durations in removing chlorpyrifos ethyl residues from grape samples at all temperatures (Akyildiz et al., 2014).

In our study, a grape sample obtained from a vineyard in Şirvan district was found to have a Malathion residue, an insecticide used in aphid control, exceeding the MRL value. However, the detected levels of the active ingredients in the remaining samples were below the MRL values, indicating an acceptable range.

Pesticide residues detected in fresh grape samples obtained from different vendors at local markets in Izmir, and determined to be grown in Salihli and Turgutlu districts of Manisa province, were evaluated based on the Turkish Food Codex and EU Maximum Residue Limits (MRLs). While 26.66% of the samples were found to be free of residues, 73.33% were contaminated with pesticide residues (Tatlı, 2006).

A study conducted to determine pesticide residues in grapes and strawberries obtained from local markets and supermarkets available to the public in the Konya region found that 38% of the total product had no residues, 10% had residues of one pesticide, 20% had residues of two pesticides, 10% had residues of three pesticides, 11% had residues of four pesticides, 9% had residues of five pesticides, and 2% had residues of six or seven pesticides (Ersoy et al., 2011). In Italy, a research conducted in 1998 and 1999 to determine pesticide residues in grapes and wines revealed residue levels ranging from 2.1% to 6.1% in grape samples, while no residues were found in wines (Cabras and Conte, 2001).

Türkiye holds a significant share in global production of fresh and dried grapes. The presence of pesticide residues above the Maximum Residue Limit (MRL), which is allowed for a long period of time, in food can lead to chronic effects and serious health problems in humans (Sternberg, 1979). Pesticide residues have been associated with various health issues, including lung diseases, skin diseases, hematopoietic system diseases, immune system diseases, digestive system diseases, kidney and urinary tract diseases, as well as reproductive system disorders such as miscarriage, low birth weight, toxemia, and postpartum bleeding (Güler and Çobanoğlu, 1994). Intensive and uncontrolled use of chemical pesticides not only affects human health (carcinogenic, mutagenic, and teratogenic effects) but also leads to the destruction of plant and animal species and harm to non-target organisms by contaminating groundwater. The elimination of certain crucial organisms involved in maintaining ecological balance can cause previously non-threatening pests to become economically harmful (Topuz, 2005)

Errors made in the use of pesticides, both in terms of quantity and application timing, can leave residues that can lead to serious health problems. Another significant issue in this regard is the potential problems we may encounter in production aimed at export. In particular, the detection of banned pesticides or the presence of residue levels exceeding acceptable limits highlights the need to take this matter seriously and address it with utmost care. Increasing efforts in this direction in our country and worldwide, and implementing serious sanctions if necessary, are necessary. Otherwise, various types of cancer, as well as many anomalies starting from birth and various health problems, cannot be prevented.

The problems that pesticides can bring in terms of environment, health, and economy are being seriously addressed in developed countries, particularly in the United States, where agricultural products are constantly subjected to inspection. With Türkiye starting the negotiation process with the EU, the protection of the environment and public health, and bringing our standards up to the level of these countries, are important issues that require the conscientious and controlled use of pesticides, and work is progressing in this direction. Residues of pesticides used for agricultural purposes significantly harm human health in the environment and food. Especially, pesticide residue levels exceeding daily acceptable limits can lead to serious health problems such as chronic and acute poisoning, teratogenic, mutagenic, and carcinogenic effects on humans (Çiftçioğlu and Issa, 2006).

According to the International Environmental Protection Agency (EPA), pesticides are defined as any substance or mixture of substances used to prevent, alleviate, eliminate, or control the adverse effects of organisms such as insects, pests, rodents, weeds, fungi, bacteria, and viruses that may cause harm to agricultural products, humans, or other living beings.

Türkiye holds an important position in grape production worldwide. However, while dried grape exports are at the desired level, table grape exports are at a low rate. One of the biggest problems in fresh grape exports is pesticide residues. This situation is particularly evident in the Dangerous, Risky, and Harmful Products list prepared by Greenpeace in Germany, where grapes exported from Türkiye to Germany rank high, indicating the seriousness of the situation (Anonymous, 2012). The current state of affairs demonstrates that agriculture conducted using chemical pesticides is not sustainable and calls for the application of alternative methods in agriculture. All these factors necessitate the restriction of chemical pesticide use and the promotion and widespread use of sustainable products that do not harm humans, animals, or plants (Gazioglu Sensoy et al., 2022).

4. CONCLUSION

Efforts should be intensified to raise awareness among producers and consumers regarding the use of agricultural chemicals, especially by limiting the use of systemic effective preparations and recommending, supporting, and implementing obligations for the use of contamination-effective pesticides when necessary. It is recommended to control the use of pesticides, which are currently being used without knowledge and inappropriately, more rigorously through stricter inspections and informative measures. In light of these findings, it is advisable for universities, research institutes affiliated with the Ministry of Food, Agriculture and Livestock, Provincial Control Laboratories, Provincial and District Directorates, as well as non-governmental organizations such as Chamber of Agriculture, to conduct coordinated efforts to increase the knowledge level and awareness of both producers and consumers. It is crucial for the health of humans and the environment to implement punitive measures at the national level or to encourage and incentivize producers to accept pesticides with low damage thresholds through various measures.

Although this study does not provide a comprehensive understanding of the extent of the residue problem in products available to the public and is not fully sufficient to make definitive judgments, it carries the characteristic of a preliminary work for comprehensive residue screenings that will be conducted with broader funds. It is important in terms of identifying the problems and demonstrating the necessity of steps to be taken for solutions.

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