Determination of Pesticide Residues in Grapes From Vineyards Implemented Good Agricultural Practice in Uşak

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Abstract: This study was conducted to determine pesticides residue levels in grapes samples which taken from vineyards implemented good agricultural practice in Uşak province in 2017 growing seasons. A total of 51 grape samples from three districts were collected. Liquid chromatography with mass spectrometry (LC-MS/MS) and gas chromatography with mass spectrometry (GC-MS/MS) devices were used for all analyses. In 45.1% of the samples taken weren’t detected any pesticide residue. In 54.9% of grape samples found residue, none of this pesticides exceeded the maximum residue limits given in Turkish Food Codex. The most common pesticides detected in grape samples with residue were spinosad, pyrimethanil and boscalid respectively. Thirteen different pesticide active substances were detected below the MRL in the samples. All pesticides detected in the samples were fungicides (85%) and insecticides (15%).

Uşak İlinde İyi Tarım Uygulamaları Yapılan Bağ Alanlarındaki Üzümlerde Bulunan Pestisit Kalıntılarının Belirlenmesi

Anahtar Kelimeler: Kalıntı, Pestisit, Üzüm, Uşak

Özet: Bu çalışma, 2017 üretim sezonunda Uşak’ta iyi tarım uygulamaları yapılan bağ alanlarında yetiştirilen üzümler üzerinde bulunan pestisitlerin kalıntı düzeylerinin belirlenmesi amacıyla yapılmıştır. Üç farklı ilçeden alınan toplam 51 adet üzüm numunesi, Sıvı Kromatografi/Kütle Spektrometresi (LC-MS/MS) ve Gaz Kromatografi/Kütle Spektrometresi (GC-MS/MS) cihazlarıyla analiz edilmiştir. Toplanan üzüm örneklerinin %45.1’inde herhangi bir pestisit kalıntısı rastlanmamıştır. Kalıntı tespit edilen örneklerin hiçbirinde kalıntı seviyesi, Türk Gıda Kodeksi’nçe belirlenen maksimum kalıntı seviyelerini aşmamıştır. Kalıntı üzüm örneklerinde tespit edilen en yaygın pestisitler sırasıyla spinosad, pyrimethanil ve boscalid olmuştur. Tespit edilen 13 farklı pestisitin %85’ini fungisitler, %15’ini de insektisitler oluşturmıştır.

1. Introduction

Although the use of pesticides in agriculture has brought many benefits to the producer, the overuse and misuse of these chemicals has led to high levels of pesticide residues on the crops. This situation is to be a problem in international market. Recently, this problem has been relatively reduced with the spread of Good Agricultural Practices (GAP). Even if the pesticides are applied in accordance with principles of GAP, residues may remain on the crops [3].

This study was conducted to determine pesticide residue levels in grape samples collected from vineyards implemented GAP in Uşak province.
2. Material and Method

2.1. Chemicals and reagents

Pesticides given in the Table 1 were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and all of them are >95% pure. Acetonitrile (MeCN), Magnesium sulphate (MgSO4), sodium acetate were analytical grade from Merck (Germany). Primary secondary amine (PSA) was from Sigma-Aldrich (England) and it also was analytical grade.

2.2. Instrumentation

An Agilent 1260 series coupled to a 6420 model triple quadruple mass spectrometer (Agilent Technologies Inc., CA) was applied for chromatographic separation. The instrument settings were gas temperature 350 °C; gas flow, 12 L min$^{-1}$; nebuliser gas, 50 psi; sheath gas temperature, 350 °C; sheath gas flow, 12 L min$^{-1}$; capillary voltage, 2000 V. Mass Hunter Quantitative analysis software (Agilent Technologies, Palo Alto, CA, v.B.05) were used for all data analyses.

The chromatographic separation was performed on a Rapid Resolution reverse phase column-C18 2.7 µm, 2.1 × 100 mm column (Agilent Technologies). The mobile phases comprised of 100% water in 5 mM ammonium formate containing 0.1% formic acid for solvent A and acetonitrile in 5 mM ammonium formate containing 0.1% formic acid for solvent B. LC and MS/MS conditions were injection volume, 5 µL; oven temperature, 55°C; flow rate of mobile phase, 0.5 mL min$^{-1}$ and total elution time, 7.5 minutes.

Gas chromatography analysis were performed using an Agilent 7890A GC system with an Agilent 5975C Series GC/MSD (Agilent Technologies), equipped with capillary column HP-5MS (30 m × 0.25 mm × 0.25 µm); electron capture.

Besides LC-MS/MS and GC-MS/MS devices, a centrifuge, centrifuge tubes up to 50 mL and 15 mL capacity, a digital balance, vortex, blender, glass GC vials (1.5 mL) were used during laboratory studies.

2.3. Collection of the samples

Grape samples were collected from a total of 51 different vineyards across three different districts of Usak province where produced the most grapes according to data of Usak Directorate of Provincial Food Agriculture and Livestock in September 2017 when was pre-harvest period. The grape samples which is material of the study were taken as 2 kg from each production field. Disposable polyethylene gloves were used to prevent contamination during collection of samples. The collected samples were sent to the laboratory on the same day without waiting.

2.4. Cleanup and extraction of the samples

The grape samples were extracted by QuEChERS method [4]. The samples were homogenized with steel blenders by shredding. 15 g of the homogenized sample was taken and placed into 50 mL falcon tube which contains 15 mL of acetonitrile with 1 % acetic acid. Afterwards, 6 g of anhydrous magnesium sulfate and 1.5 g of sodium acetate is added into falcon tubes and centrifugated for 5 min. For cleanup stage, 4 mL of the sample were transported into 15 mL falcon tubes which contain 1200 mg of anhydrous MgSO4 and 400 mg primary secondary amine (PSA) and centrifuged. Then, 1 mL of the extracts was transferred into vialas and kept in a freezer [4]. The vials were taken from the freezer, waited under room conditions and analyzed by LC-MS/MS and GC-MS/MS. Figure 1 represents a schematic diagram illustrating the workflow of analysis.

![Workflow diagram for the analysis](image)

**Figure 1.** Workflow diagram for the analysis (Adapted from [5])

2.5. Analysis of the samples

Pesticide analyses of the samples were performed in three different laboratories accredited for all analytical methods. It was used liquid chromatography with mass spectrometry (LC-MS/MS) and gas chromatography with mass spectrometry (GC-MS/MS) devices for all analyses.

3. Results and Discussion

The matrix-matched calibration curves of all pesticides were found as linear ($R^2>$ 0.99) in calibration limits. Concentration ranges, analytical functions, limit of quantification (LOQ) and MRLs of the pesticides detected on the samples are present Table 1.

Twenty three samples (45.1%) of the total of 51 grape samples had no detectable residue, while 28 samples (54.9%) contained residues lower than maximum residue limits given in Turkish Food Codex.
so found any pesticide residue in mers’ training and monitoring st grapevine -Lobesia botrana as detected a pesticide residue above the samples are shown in Figure 3. In 23 of the samples analyzed were detected no pesticides residue. Simi pesticides residue. Folpet was the most detected fungicide residue. Whereas in this study, it was more residues more than fungicide residues in the same study. It was found insecticide which commonly used against grapevine moths (Lobesia botrana Den. & Schiff.) in vineyard. In vineyards from Denizli, Izmir and Manisa provinces, a study conducted by [6] was detected lambda-cyhalothrin as the most common pesticide residue. In the same study, it was found insecticide residues more than fungicide residues in the samples. Whereas in this study, it was more fungicide residue. Folpet was the most detected pesticide in the study conducted by [8]. One of the grape samples analyzed had ten different pesticide residue. Similarly, [9] reported that two samples of grapes had nine pesticide residue in their study. This number found as seven per sample by [8].

In 23 of the samples analyzed were detected no residue, one sample had one residue, four samples had two residue, five samples had three residue, eight samples had four residue and ten samples had four and more residue. The residue percentage of the samples are shown in Figure 3.

Similarly, [6] analyzed grape samples taken from vineyards implemented integrated pest management (IPM) and organic farming in Manisa, Denizli and Izmir provinces, Turkey and detected no pesticide residue. In same region, a study conducted by [7] wasn’t also found any pesticide residue in grape samples. 27 out of the total of 28 grape samples detected residue contained residues of two or more pesticides. As a result of analysis, 13 different pesticide active substances were detected. These pesticides, MRLs and concentration ranges are presented in Table 1. All the detected residues below the MRL.

All pesticides detected in the samples were fungicides (85%) and insecticides (15%). The frequency of the most detected pesticides in the grape samples are given in Figure 2. As shown in the graph, the most common pesticides detected in grape samples with residue were spinosad, pyrimethanil and boscalid respectively. Spinosad that found in 27 of 28 samples with residue is a bio-insecticide which commonly used against grapevine moths (Lobesia botrana Den. & Schiff.) in vineyard. In vineyards from Denizli, Izmir and Manisa provinces, a study conducted by [6] was detected lambda-cyhalothrin as the most common pesticide residue. In the same study, it was found insecticide residues more than fungicide residues in the samples. Whereas in this study, it was more fungicide residue. Folpet was the most detected pesticide in the study conducted by [8]. One of the grape samples analyzed had ten different pesticide residue. Similarly, [9] reported that two samples of grapes had nine pesticide residue in their study. This number found as seven per sample by [8].

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### Table 1. Active substances detected in grape samples

<table>
<thead>
<tr>
<th>Active substance</th>
<th>No. of samples detected residue</th>
<th>Frequency (%)**</th>
<th>Range (mg.kg⁻¹)</th>
<th>MRL*** (mg.kg⁻¹)</th>
<th>LOQ (mg.kg⁻¹)</th>
<th>Analytical function</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoxystrobin</td>
<td>2</td>
<td>7.1</td>
<td>0.029-0.039</td>
<td>2</td>
<td>0.01</td>
<td>y=7057.8+10503.74x</td>
<td>0.998</td>
</tr>
<tr>
<td>Boscalid</td>
<td>20</td>
<td>71.4</td>
<td>0.059-2.737</td>
<td>5</td>
<td>0.02</td>
<td>y=623.93+727.96x</td>
<td>0.996</td>
</tr>
<tr>
<td>Bupirimate</td>
<td>1</td>
<td>3.6</td>
<td>0.012</td>
<td>1.5</td>
<td>0.01</td>
<td>y=505.46+3563.53x</td>
<td>0.998</td>
</tr>
<tr>
<td>Cyproconazole</td>
<td>10</td>
<td>35.7</td>
<td>0.024-0.31</td>
<td>3</td>
<td>0.02</td>
<td>y=2281.86+3560.98x</td>
<td>0.999</td>
</tr>
<tr>
<td>Famoctizone</td>
<td>2</td>
<td>7.1</td>
<td>0.03-0.25</td>
<td>2</td>
<td>0.01</td>
<td>y=66.25+204.01x</td>
<td>0.995</td>
</tr>
<tr>
<td>Fenhexamid</td>
<td>16</td>
<td>57.1</td>
<td>0.097-0.43</td>
<td>15</td>
<td>0.02</td>
<td>y=800.92+285.54x</td>
<td>0.996</td>
</tr>
<tr>
<td>Fludioxonil</td>
<td>14</td>
<td>50</td>
<td>0.015-0.57</td>
<td>5</td>
<td>0.01</td>
<td>y=116.45+87023x</td>
<td>0.997</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>4</td>
<td>14.3</td>
<td>0.017-0.182</td>
<td>1</td>
<td>0.01</td>
<td>y=190.72+34797x</td>
<td>0.998</td>
</tr>
<tr>
<td>Iprodione**</td>
<td>1</td>
<td>3.6</td>
<td>0.041</td>
<td>20</td>
<td>0.01</td>
<td>y=264.78+12651x</td>
<td>0.998</td>
</tr>
<tr>
<td>Pyrimethanil**</td>
<td>24</td>
<td>85.7</td>
<td>0.025-0.77</td>
<td>5</td>
<td>0.01</td>
<td>y=2988.26+177326x</td>
<td>0.998</td>
</tr>
<tr>
<td>Spinosad</td>
<td>27</td>
<td>96.4</td>
<td>0.011-0.18</td>
<td>0.5</td>
<td>0.01</td>
<td>y=2481.31+213837x</td>
<td>0.997</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>6</td>
<td>21.4</td>
<td>0.019-0.218</td>
<td>0.5</td>
<td>0.01</td>
<td>y=4011.26+183367x</td>
<td>0.998</td>
</tr>
<tr>
<td>Triadimenol</td>
<td>13</td>
<td>46.4</td>
<td>0.021-0.22</td>
<td>2</td>
<td>0.01</td>
<td>y=29.83+54464x</td>
<td>0.998</td>
</tr>
</tbody>
</table>

**Active substances detected by GC-MS/MS. ** Frequency in samples with residue. *** Value of Turkish Food Codex.

**Figure 2.** The frequency percentage of pesticide residue detected below MRL in the samples

**Figure 3.** Percentage of the number of residue in the samples

### 4. Conclusion

This study was aimed to determine the level of pesticides residue in grape samples collected at preharvest. According to the analysis results, the most detected pesticide was spinosad which is a biological insecticide. None of 51 grape samples collected was detected a pesticide residue above MRL. This situation is one of the most important benefit of GAP which is a system adopting IPM. As it was found four and more pesticide residue in 18 of the grape samples, we have considered that practices, such as farmers’ training and monitoring residue at preharvest, need to be continued.
References


