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ANALYSIS OF PESTICIDES IN THE COMPOSITION OF GRAPES

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Abstract. The paper deals with the three grape varieties - merlot, savignon cabernet and bayanshire from the grape sites of the Ganja-Gazakh region of Azerbaijan. The determination of the residual amount of pesticides in these varieties carried out in the laboratory of the Azerbaijan Institute of Food Safety. The qualitative determination of nitrogen-containing fungicides containing a phenyl residue as well as the quantitative determination of the mospilan insecticide is made using the example of azox and ridomil gold by gas chromatography according to the approved methods. Analyzes were performed on the GCMS 7820A analyzer.

Keywords: *analysis, insecticide, chromatography, quantity, fungicide.*

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1. Introduction

Viticulture and winemaking are one of the important industries in Azerbaijan. Industrial production of grapes is dangerous in terms of man-made impacts on the country's ecology (Abbasov *et al*., 2002). The main reason for the technogenic influence is that the vineyard is cultivated for a long time without rotation on the plots and is subjected to a constant load of pesticides and other chemicals (Nakajima *et al*., 2001). As a result there is a decrease in the energy potential of plantings a decrease in productivity and product quality as well as in terms of food safety and other environmentally negative effects. From this point of view modern insecticides and fungicides pose a great danger (Movsumov & Guliyeva, 2010). It should be noted that the ecological and toxicological aftereffect of these pesticides which are characterized by a high protective effect is insufficiently studied (Bankina, 2002). They have the increased toxicity of most of them and a low consumption rate when used in vineyards (Dubtsov, 2001). The ecological and toxicological hazard of most fungicides and insecticides is the prolongation of their decay to safe compounds and the formation of intermediate half-life products the toxicity of which may exceed the drug itself (Hajiyeva *et al*., 2017a; Bassil *et al*., 2007). Gas and liquid chromatography methods used in the work to identify toxic residues of the determined drugs and their metabolites allow obtaining complete and objective information on the sanitary and hygienic indicators of this material (Borgio *et al.*, 2011; Hajiyeva *et al.*, 2017b).

Grapes are one of the most valuable food products (Ganiyev & Nedorezkov, 2006). The berries of fresh grapes contain easily digestible sugars - glucose and fructose, organic acids - malic, tartaric, citric, succinic, etc., mineral salts of potassium, calcium, sodium, phosphorus, manganese, cobalt, iron, trace elements and phenolic substances (Lamberth *et al*., 2013).

The processing of grapes for wine, juices, preserves, marmalades the preparation of raisins and other products significantly expand its sphere of consumption (Orlov, 2002).

The possibility of long-term storage the high content of sugars useful for the body and high calorie content make these products strategic (Shikhlinskiy, 2006).

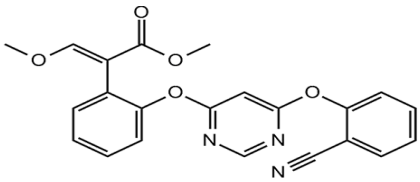
The Ganja-Gazakh zone is one of the main producers of grapes and its processed products in Azerbaijan.

2. Data analysis and processing

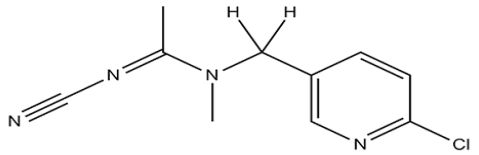
The material for analysis was selected in the vineyards of specialized farms of one of the main viti cultural zones of the region (Shamkir, Gazakh and Ganja regions) against the background of ecological and toxicological monitoring. The objects of research are three grape varieties merlot, savignon cabernet and bayanshire. Instrumental work to determine the residual amounts of pesticides. In the specified material were performed in the People's Reference Laboratory of the Azerbaijan Institute of Food Safety. In the objects under study, the content of nitrogen-containing fungicide preparations containing a phenyl residue using the example of azox and ridomil gold was determined by gas chromatography (GC) according to approved methods as well as the mospilan insecticide. The active ingredients of these pesticides in the composition of azoxa-azoxytrobin in the composition of ridomil gold -mancozeb and metalaxil in the composition of mospilan - asetamiprid. Due to the lack of a standard for the determination of mancozeb, metalaxyl, i.e. another active substance in grapes was determined.

Azoxystrobin [methyl (E) -2- {2- [6- (2-cyanophenoxy) pyrimidin-4-yloxy] phenyl} -3-methoxyacrylate] is a fungicide of systemic and contact action, has a long-term protective effect. The drug is highly effective against pathogens of downy mildew and powdery mildew as well as against races of the pathogen resistant to triazole and metalaxyl derivatives.

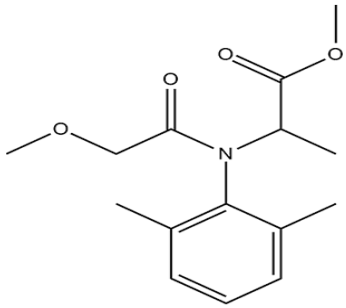
This active ingredient of pesticides a fungicide from the strobilurin class is used in agriculture (including in a mixture with other active ingredients) to combat various plant diseases is a fungicide of systemic and contact action has a long-term protective effect (Colović *et al*., 2013). The drug is highly effective against pathogens of false and powdery mildew, as well as against races of the pathogen resistant to triazole derivatives and metalaxyl.



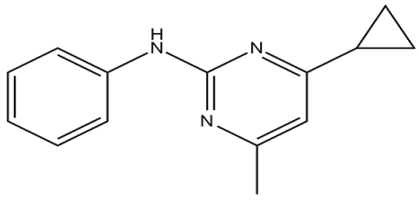
Acetamiprid [N1-methyl-N1 - [(6-chloro-3-pyridyl) methyl] -N2-cyanacetamidine] is a chemical active ingredient of pesticides (neonicotinoid), used in agriculture and private household plots to combat harmful insects. Acetamiprid is a white crystalline substance.At pH 4-7 it is hydrolytically stable, at pH 9 and 45°C it gradually decomposes. Stable in sunlight. Melting point 98.9°C; vapor pressure at 25° C <1,10-6 Pa (<7.5,10-9 mm Hg); solubility in water 4200 mgL^{-1}; let's dissolve in acetone, ethanol, methanol, acetonitrile, dichloromethane, chloroform, tetrahydrofuran.



Metalaxyl [N- (2,6-dimethylphenyl) -N- (2-methoxyacetyl) alanine methyl ester] is a pesticide, systemic fungicide from the class of phenylamides, acylalanines, effective against pathogenic organisms belonging to the order Peronosporales. White or beige crystals of this substance are stable in acidic and neutral environments: at 20°C and pH = 1 50% hydrolysis occurs in 200 days at pH 9 - in 115, at pH> 10 - in 12 days. Decomposes at 300 ° C. Medium resistant to light. Soluble in most organic solvents. Volatility is negligible. Metalaxyl can exist as two isomers R and S that differ greatly in biological activity. The most active is the R isomer. Preparations based on it received the prefix "gold". Recently this isomer as an active ingredient has become known as mefenoxam.



Cyprodinil [4-cyclopropyl-6-methyl-N-phenyl-pyrimidin-2-amine] is a pesticide active ingredient a systemic fungicide from the anilidopyrimidine class. It is effective against a number of diseases of fruit crops and grapes. It is a beige powder with a low odor. The chemically pure active ingredient is a creamy crystalline compound with a smell. It is resistant to hydrolysis (at pH = 4-9 half-decomposition period is more than 1 year). Photolytically quickly decomposes in water (DT50 = 0.4-13.5 days). In plants it is metabolized by hydroxylation of the 6-methyl group, phenyl and pyrimidine nuclei. The drug has less prophylactic effect than captan and mancozeb and weaker eradicating than sterol inhibitors against apple scab. It is active at low temperatures (+ 3°C). It is not washed off by rain already 2 hours after treatment. Effective at the beginning of the season against scab of fruit crops, has a partial effect on powdery mildew alternaria and moniliosis of fruit. Effectively inhibits the growth of mycelium of various pathogens, including those resistant to fungicides from the groups of imidazoles, triazoles, etc. The substance quickly penetrates into plant tissues, has good acropetal and laminar translocation. Inhibits the biosynthesis of methionine. It has a systemic (within 7-10 days) and therapeutic (within 36 hours if the treatment is carried out when the first signs of infection appear). Does not cause resistance to pathogens, subject to strict adherence to the recommendations.



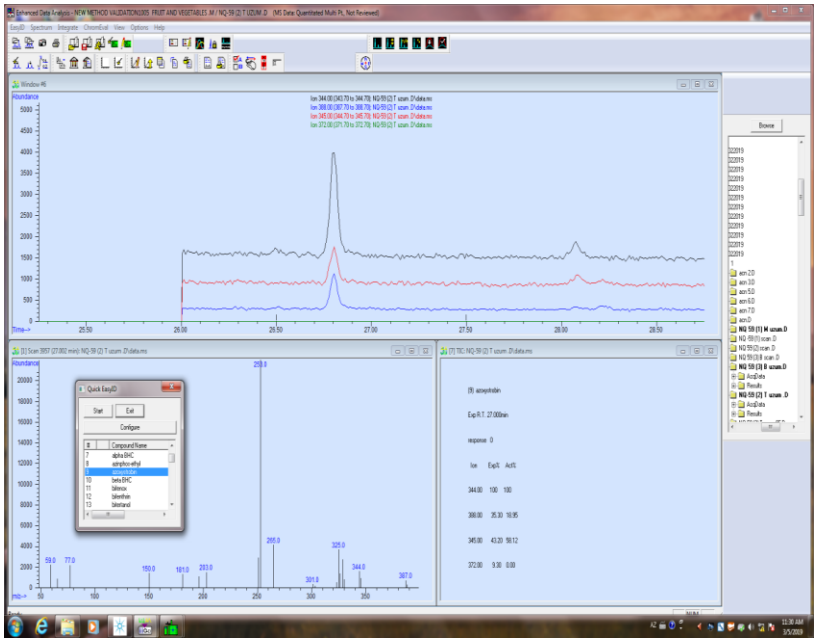


Figure 1. Chromatogram and mass spectrum of azoxytrobin sample of the merlot variety from a vineyard on the territory of the Shamkir region

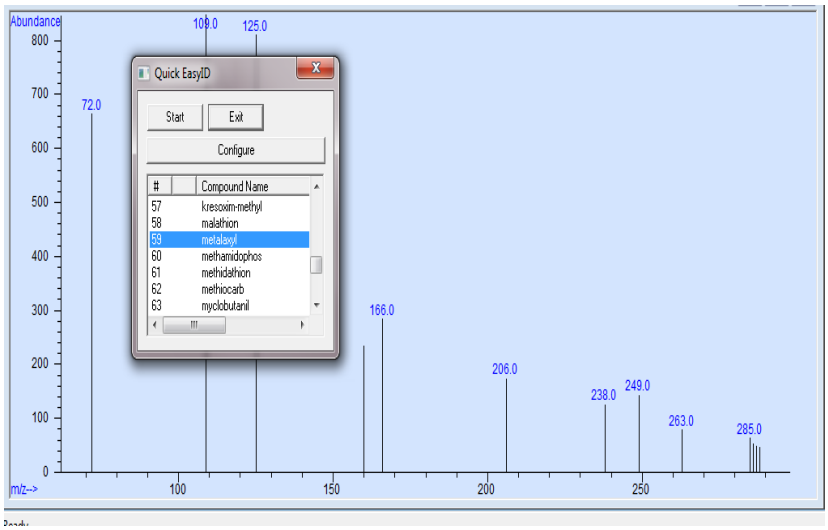


Figure 2. Chromatogram and mass spectrum of metalaxyl sample of the merlot variety from a vineyard on the territory of a winery in Shamkir region

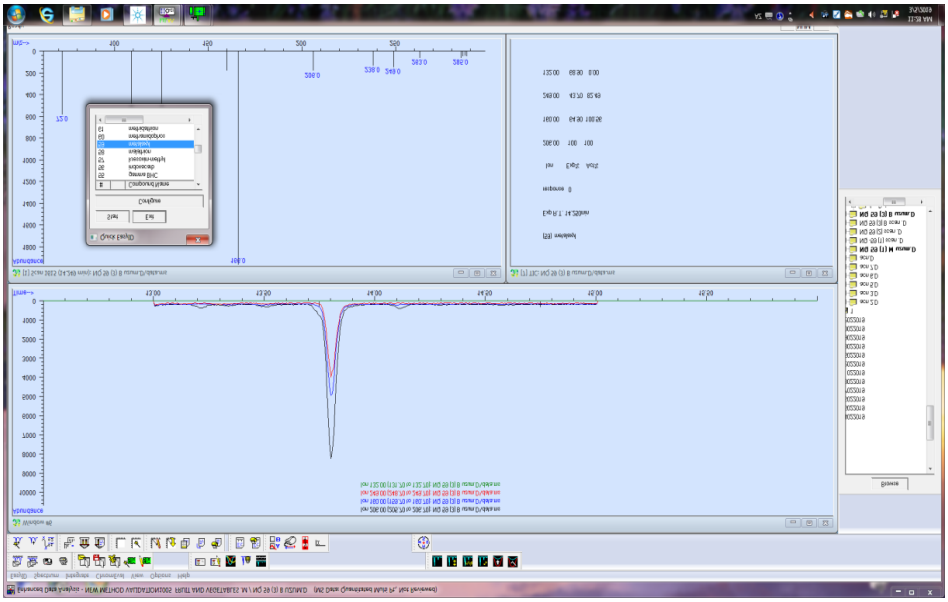


Figure 3. Chromatogram of azoxytrobin sample of the cabernet sauvignon variety from a vineyard on the territory of a private production of Gazakh region

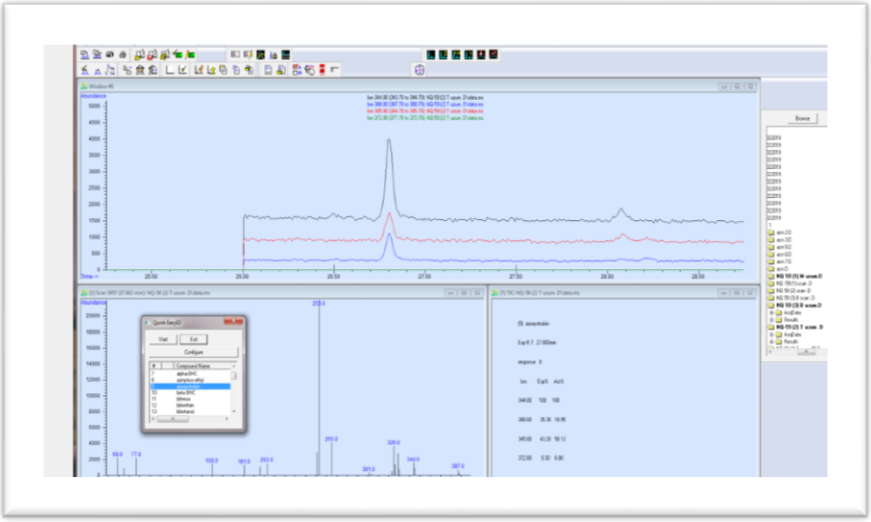


Figure 4. Chromatogram graph for the determination of acetamiprid in a sample of bayanshire variety from a vineyard on the territory of the Ganja winery by gas-liquid chromatography

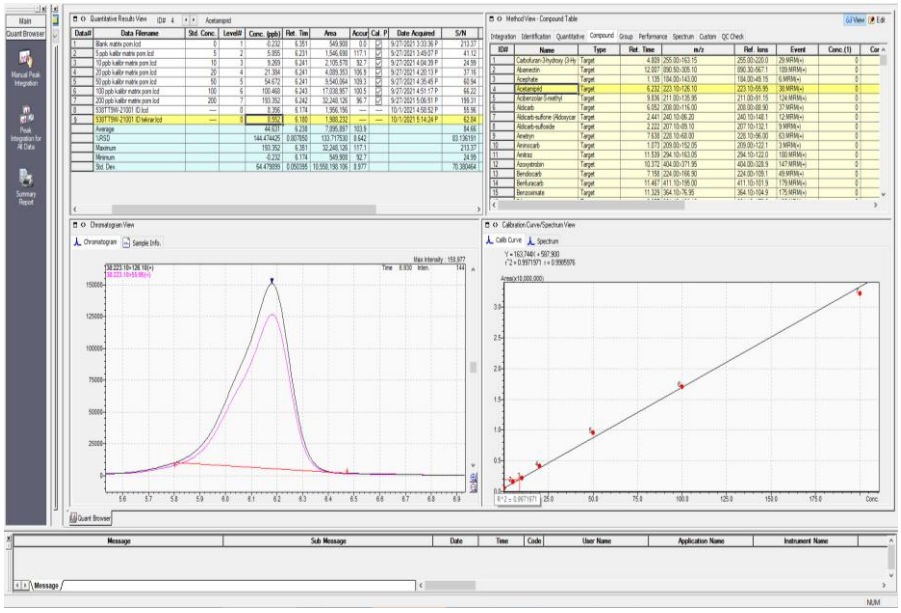


Figure 5. Chromatogram graph for the determination of cyprodinil in a sample of bayanshire variety from a vineyard on the territory of a Ganja winery by gas-liquid chromatography

The analysis of samples according to the presented methods allows for a qualitative analysis of these fungicides and a quantitative determination of the insecticide residue in the grapes (Fantke *et al*., 2012).

In 2018 a qualitative analysis of Merlot varieties was carried out and cabernet savignon by gas chromatography, and in 2019 quantitative analysis of bayanshire variety by gas-liquid chromatography. These analyzes were performed on an Agilent 7820 A gas-liquid chromatography instrument (USA).

The sample is homogenized. After homogenization we add a part to the centrifuge tube. Due to the presence of 80% water in the composition, we do not add water. Add 10 ml of acetonitrile to the sample. Close the centrifuge and turn it on for one minute.

4g of $MgSO_4$, 1g of NaCl, 1 g of trinitrate citrate dihydrate, 0.5 g of disodium hydrocitrate sesguigitrate buffer-salt mixture were added to the resulting suspension. Vortex vigorously for one minute. After that stir in a centrifuge for five minutes. Add 6 ml of an aliquot of acetonitruleic phase to the resulting solution. We move it in the centrifuge. The solution is isolated and from the pure extract we take 1 ml. To increase the acidity add 10 µl of formic acid solution. Switch to avto sample mode and start chromatographic analysis. As a result azoxytrobin and metalaxyl were found in the merlot grape variety from the vineyard in the Shamkir region winery in the cabernet sauvignon grape variety in the vineyards of the private territories of the Gazakh region and the bayanshire variety on the territory of the Ganja winery. Below are the chromatograms for the detection of the listed compounds.

The amount of acetamiprid in the bayanshire variety was 8.55 ppb using quantitative analysis. The amount of cyprodinil in the bayanshire variety by quantitative analysis is 50.545 ppb. Below is a list of grape samples that should be used to determine pesticides:

Table 1. Detected pesticides in grape varieties

Varieties	Detected pesticides
Merlot	azoxytrobin, metalaxyl
Savignon cabernet	azoxytrobin
Bayanshire	acetamiprid, cyprodinil

As a result of the qualitative analyzes azoxytrobin and metalaxyl were found in the composition of the merlot grape variety the presence of azoxytrobin in the savignon cabernet variety and the presence of acetamiprid 8.55 ppb and cyprodinil 50.45 ppb in the bayanshire variety using quantitative analysis.

Also in the studied grape varieties the analysis of the physicochemical indicators carried out.

At first the wort was obtained from the grapes of the studied varieties. Initially after peeling the grapes well all the spoiled berries were removed. After grinding, the juice was separated to determine the indicators.

In winemaking they are guided not by the acid content in berry juice but by the pH value. The pH level indicates the concentration of active acids in grape juice and is determined in laboratory conditions using free hydrogen ions. A high pH indicates a low concentration of active acids a low pH indicates a high one. The pH level indicates the presence of palatable and non-volatile acids.The pH was measured with a pH meter. The hydrogen index for the Merlot variety was -3.38, for the Savignon Cabernet variety -3.39 and for Bayanshire -3.29.

Total acidity shows the total content of titratable acids that is the content of all acids possible in bulk chemical analysis including volatile ones.To determine the total acidity 10 ml of grape juice and 5 drops of bromine thymol blue were added to the flask and titrated with 0.1 N NaOH solution. The used alkali solution was multiplied by a factor of 0.75 and the total acidity was determined from the table. For the Savignon Cabernet variety the total acidity index is 5.32, for Bayanshire the total acidity index is 7.57 and for the Merlot variety the total acidity index is 4.9.

To determine the sugar content the grape must density was first determined by a hydrometer-sucrometer type AON with a range of 0-25%. The test sample was taken from bunches of grapes from different vines in order to obtain averaged data. The juice for measurement should be transparent let the juice settle for 1-2 hours. It was calibrate the hydrometer to a temperature of 20^0 C. If the temperature of the juice would be different then it would be necessary to make an amendment of 0.0002 for each degree of temperature. With a decrease in temperature the density increases and with an increase vice versa. Pour juice into the vessel so that the hydrometer can float freely in it without touching the bottom and at the same time the level of juice does not reach the top of the vessel. We carefully lower the hydrometer into it so that it does not touch the walls, and we take the hydrometer readings at the lower liquid level (lower meniscus) for the accuracy of the readings, the eye level should be at the height of the juice-air border. According to the density of the juice we determine its sugar content according to the corresponding table.

Table 2. Physicochemical indicators of the grape varieties merlot, savignon cabernet, bayanshire

Varieties	Total acidity g/l	Sugar content, g/dm^3	Density, g/dm^3	pH
Savignon cabernet	5.32	239	1.1	3.39
Bayanshire	7.57	210	1,089	3,29
Merlot	4,9	228	1.096	3,38

The density index for the savignon cabernet variety is -1.1 and accordingly this density is the sugar content – 239, the density index for the bayanshire variety is -1.089 and accordingly this density the sugar content is 210, for the merlot variety the sugar content is 1.096, the sugar content is 228.

The detection of azoxytrobin and metalaxyl in the merlot variety azoxytrobin in the savignon cabernet variety, acetamiprid and cyprodinil in bayanshire does not negatively affect the physicochemical characteristics of these varieties (Table 2).

3. Conclusion

In the paper three grape varieties - merlot, savignon cabernet and bayanshire from the grape sites of the Ganja-Gazakh zone of Azerbaijan are studied, some pesticides are determined in the considered samples. Analysis of the quantitative determination of the mospilan insecticide is carried out in the considered samples. Qualitative determinations of nitrogen-containing fungicides are made containing a phenyl residue, using the example of azox and gold ridomyl, as well as a quantitative determination of the insecticide mospilan.

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