Safety evaluation of the use of one- and two-component fungicides to protect grapes

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Abstract. Viticulture and winemaking are traditional industry of agriculture in the southern regions of the Russian Federation, which playing an important role in their economy. A rich and undamaged crop is almost impossible to grow without plant protection measures. Preparations used to spray plants are by no means harmless, and do not always completely disintegrate before harvesting. The objective of the work was to study the dynamics of degradation of fungicides of systemic action, belonging to different classes, in grapes. The two-component preparation was a combination of 120 g/l difenoconazole + 60 g/l tetraconazole (MEC). The one-component preparation contained 250 g/l of cyprodinil (EC). The work was done in the Krasnodar region for 2 years. The half-lives of difenoconazole and tetraconazole were about 5 days, cyprodinil 2.7 and 4 days, 1 and 2 years, respectively. The concentration of difenoconazole and tetraconazole went down to below the MRL in the first year of the study, two weeks after the last spraying, and in the second – after three weeks, which was most likely caused by the prevailing weather conditions – " dangerous phenomenon – intense heat". At the time of harvest, no residual amounts of difenoconazole, tetraconazole and cyprodinil were found, which indicates the safety of the products obtained for consumers.

1 Introduction

Viticulture and winemaking are activities that are technologically interconnected and have the potential for dynamic development. These are traditional industry of agriculture in the southern regions of the Russian Federation, which play an important role in their economy. In 2017-2021, the gross grape harvest in Russia increased by 17.5%, and the area of vineyards in the country increased by 6.8% [1].

One of the main directions of the state policy in the field of viticulture and winemaking is: the development of scientific research, scientifically technical and innovative activities in the area of viticulture and winemaking, including activities for quality control of viticulture products and winemaking products. Federal Law "On Viticulture and Winemaking in the Russian Federation" dated 27.12.2019 N 468-FL (latest edition).

All over the world, the technology of agricultural production involves the use of a large number of different pesticides, what is a necessary condition for increasing the crop and ensuring its quality. At the same time, the total amount of pesticides used during the growing

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season can reach 55 g/ha, and the number of treatments tends to 10-12, and sometimes even more. Moreover, vegetables, fruits and berries are treated with pesticides during storage and transportation, and in such conditions, there is always a risk of contamination of these products with pesticide residues and their subsequent ingestion into the human organism. Many studies have established that pesticides, when systematically ingested into the human organism, can cause various negative effects. So, the level of pesticide residues in food is strictly regulated in each country. This index is one of the main ones from the point of view of food safety. An important instrument in preventing the negative effects of the use of pesticides on the population is the monitoring of their toxic residues in ecological objects, crop production, feed and food. That monitoring allows you to accumulate data on the level of contamination of products, assess its size, identify the most dangerous pesticides and sources of their ingress into products, as well as develop recommendations to reduce the risk of pesticides entering the human organism.

Different types of diseases damage grapes during the entire period of growth and fruiting. As a result, the productivity decreases, the quality of berries deteriorates, as well as the growth and development of plants. Cultivation of the same crop leads to the accumulation of an infectious background, so the fight against them should be carried out regularly, starting with the laying of the plantation.

It is almost impossible to grow a rich and undamaged harvest of berries without special measures to protect shrubs. They can be both destructive and preventive, mechanical, chemical and biological. Chemical methods of disease control are considered by many to be the most effective. It is more expedient to use systemic fungicides, since they have not only a protective prophylactic, but also a therapeutic effect. The results of pesticide processing in the cultivation of grapes can be not only possible environmental contamination, but also the presence of residual amounts in grapes, wort and wine, a negative effect on the fermentation and organoleptic characteristics of wine, as well as a decrease in sanitary and hygienic quality and toxicological effects on the consumer.

The objective of the work was to study the dynamics of degradation of systemic fungicides – one-component and two-component, belonging to different classes, on grapes.

These fungicides are directed at combating a whole complex of grape diseases. The preparations penetrate the plant within 2 hours after spraying, and the period of protective action is 1-3 weeks after that, depending on the phytosanitary situation.

Difenoconazole and tetraconazole are systemic fungicides from the class of triazole derivatives. Triazoles are the largest group of fungicides, belongs to the class of azoles.

Difenoconazole surpasses most preparations in the spectrum of action on phytopathogens, the fungicidal activity of him is stable during the most vulnerable period of plant development at the beginning of the growing season. It is used against a wide range of pathogens from the classes of ascomycetes, basidiomycetes, deuteromycetes. Another feature of difenoconazole is the softness of the action on the cultivated plant. Difenoconazole penetrates into plant tissues, completely inhibits the growth of subcuticular mycelium, reduces the level of sporulation of the pathogen. The effect does not depend on weather conditions, however, at temperatures of 12 °C and below, the effectiveness of decreases.

Tetraconazole is a systemic fungicide with a strong protective and destroy effect against pathogens of vegetative organs of plants. It is absorbed by roots and leaves. The most effective of all triazoles, it also acts in the form of vapours. It has a prolonged action. Suppresses the biosynthesis of ergosterol. Quickly penetrating through the cuticle (wax layer) of the plant, tetraconazole is uniformly distributed inside it without accumulation in certain parts. Characterized by translaminar activity, it moves into growing tissues. The biosynthesis of gibberellins is not affected and therefore does not deform young plant tissues.

Cyprodinil is a systemic fungicide from the class of anilidopyrimidines. Effectively inhibits the growth of mycelium of various pathogens, including those resistant to fungicides

from the groups of imidazoles, triazoles and another. It quickly penetrates into plant tissues, has good acropetal and laminar translocation. Inhibits the biosynthesis of methionine. It has a systemic (for 7-10 days) and therapeutic (for 36 hours, if the treatment is carried out when the first signs of infection appear) effect [2].

2 Materials and methods

The work was carried out in the Krasnodar region for two years.

The two–component preparation was a mixture of 120 g/l difenoconazole + 60 g/l tetraconazole (the preparative form is a microemulsion concentrate). The one–component preparation contained 250 g/l of cyprodinil (the preparative form is an emulsion concentrate).

To study the degradation dynamics of a two-component fungicide, a four-fold treatment was carried out with a norm of 0.7 l/ha of the preparation, according to active substances: 84 g/ha of difenoconazole, 42 g/ha of tetraconazole. To study the degradation dynamics of a single-component fungicide, three-time spraying was carried out, with a rate of application of 2.1 l/ha for the preparation, according to the active substance: 525 g/ha of cyprodinil. Spraying was carried out using a manual knapsack sprayer "Virolux" (Germany). All the work was carried out in 4 replicates.

Samples were taken separately from each replicate of the experiment, as well as from control variants untreated with pesticides. The selected samples were frozen at a temperature of - 18°C and stored in the freezer at the same temperature.

The analysis of samples for the content of difenoconazole was carried out in accordance with MG 4.1.2784-10. The detection limit of difenoconazole in berries is 0.02 mg/kg, in juice 0.01 mg/kg. The analysis of samples for tetraconazole content was carried out in accordance with MG 4.1.3211-14. The detection limit of tetraconazole in berries is 0.20 mg/kg, in juice 0.25 mg/kg. The analysis of samples for the content of cyprodinil was carried out according to MG 4.1.2301-07. The detection limit of cyprodinil in juice is 0.02 mg/kg. The quantitative determination of difenoconazole, tetraconazole and cyprodinil was carried out on a gas chromatograph “Crystal 2000M”.

3 Results and discussion

The residual amounts of pesticides in agricultural products and products of their recycling are strictly controlled by manufacturers. Products exceeding the maximum residual level (MRL) are not allowed to be sold to the community. The use of food raw materials with a high content of pesticides for food production is prohibited in cases where the content of toxicants in the final product cannot be reduced to acceptable concentrations by industrial, culinary and technological processing.

The presence of residual amounts depends on various factors, including which preparations were used and in which formulations, the rate of use used, the number of treatments, the time between processing and harvesting, climatic factors [3].

The results of the analysis of the content of fungicides in berries and grape juice are presented in Table 1 and Figure 1.

Table 1. The content of residual amounts of active substances of a two-component fungicide in grapes (mg/kg).

Days after treatment	first year*		second year	
	difenoconazole	tetraconazole	difenoconazole	tetraconazole
Treatment day	0.96	0.43	2.51	1.09
10 days	0.20	0.28	1.08	0.50
20 days	0.09	0.15	0.57	0.32

30 days	Not detected	Not detected	0.12	0.11
40 days (harvest)	Not detected	Not detected	Not detected	Not detected

* first year - grapes, planting 2010, grade "Riesling Rhenish";
second year - grapes, planting 2004, grade "Chardonnay".
MRL of difenoconazole 0.5 mg/kg, MRL of tetraconazole 0.25 mg/kg.

The half-lives of difenoconazole and tetraconazole were about 5 days, but in the first year of the study the decomposition rate was higher, which is most likely due to the prevailing weather conditions. A significant shortage of precipitation and elevated temperatures in June contributed to the development of soil drought. The maximum air temperature is above 30 °C and reached 39-40 °C for several days in the third decade of June (a dangerous phenomenon of "intense heat"). In the first decade of July, soil drought reached the criteria of "dangerous phenomena". There were no extreme weather conditions in the second year. The content of active substances decreased to a level below MRL in the first year of the study two weeks after the last treatment, and in the second – three.

The two-component preparation was a microemulsion, which, unlike an emulsion with only kinetic stability, is a thermodynamically stable system, which allows for a protective effect even in extremely hot weather conditions.

When analyzing the critical scheme of fungicide use in Indian viticulture, it was found that the pre-harvest intervals for tetraconazole and difenoconazole were 12.5 and 25.5 days at recommended doses and 28.5 and 38.5 days at double doses, respectively [4].

Scientific research has shown that the behavior of difenoconazole obeys first-order kinetics with a dissipation rate of 0.294 days. As in our experiment, the estimated half-life was 4.494 days [5].

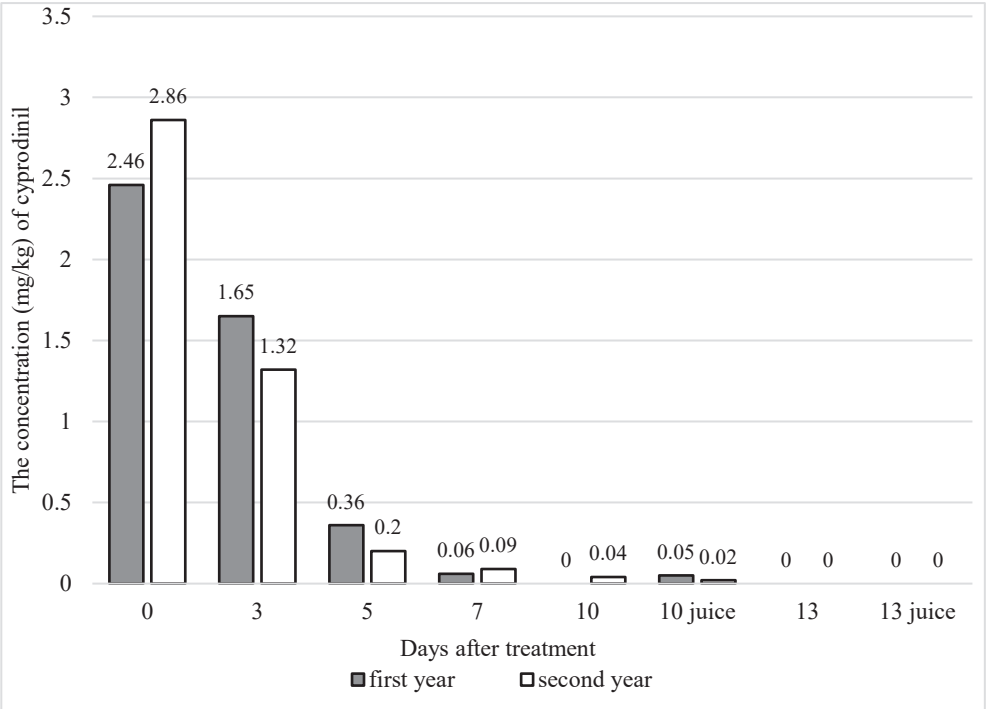

Fig. 1. The content of residual amounts of the active substance of a single-component fungicide in grapes.

The half-life of cyprodinil was 2.7 and 4 days, in the first and second years, respectively, depending on weather conditions. On all days of sampling, the residues were below MRL (5.0 mg/kg), which indicates safety for consumers.

It was found that 21 days after application, the residual amounts of cyprodinil in table grapes were below the maximum residual level (0.05 mg/kg) [6].

Correct use of wine-making processes can affect the decomposition of pesticide residues, however, it has been noted that crushing grapes does not affect the disappearance of, for example, cyprodinil. Whereas, the residual amounts of tetraconazole in grapes on 21 days after treatment were very low and were not detected [7, 8].

4 Conclusions

No residual amounts of difenoconazole, tetraconazole and cyprodinil were found in the grape crop (berries and juice).

One-component and two-component fungicides with these active substances can be used to effectively protect vineyards. The use of a mixture of several active substances in one preparation helps to avoid resistance in pathogens. When using them in the recommended application rates, in compliance with all regulations, the resulting products can be considered safe from the point of view of health risks owing to the toxic effect of residual pesticide content.

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