THE STUDY OF THE CONTENT OF RESIDUES OF “BATRIDER” INSECTICIDE IN THE HARVEST OF CHERRY AND BLACK CURRANT

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Abstract

The technology of agricultural production involves the use of a significant amount of various pesticides, which is necessary to increase the yield and ensure production quality. In 2014, the consumption of chemical protective means in Russia amounted to 54.3 thousand tons, which is 97.3\% of the total amount of pesticides consumed. Many specialists consider chemical methods of pest control to be the most effective. However, we should not forget that the preparations used for treating plants are not harmless and do not always completely decompose before harvesting. The aim of the present work was to study the dynamics of residual quantities of alpha-cypermethrin, imidacloprid, and clothianidin, the active ingredients (125+100+50 g/l) of suspension concentrate (SC) of “Batrider” insecticide in fruits and juice of cherry and black currant after treatment of the vegetative culture. The determination of alpha-cypermethrin, imidacloprid, and clothianidin was performed by gas-liquid and high-performance liquid chromatography. The study of decomposition dynamics of the active ingredients of the insecticide revealed that their content was rapidly decreasing. On the 20th day after application, it already was at ½ of Maximum Residue Limit (MRL) and further they were not detected in any of the studied cultures in all of the considered climatic zones. Thus, the use of “Batrider” insecticide (SC) provides reliable protection to crops and allows one to get high-quality and safe products that can be consumed or used for production of juices and baby food.

Keywords: Insecticide, active ingredient, alpha-cypermethrin, imidacloprid, clothianidin, stonefruit and berry crops, chromatography
I. Introduction

An important component of the competitive agro-industrial complex is a developed fruit and vegetable industry. Russia has sufficient resource potential in this area. In 2008, over 12 million tons of vegetables and about 3 million tons of fruits were grown. At the same time, the provision of the population with own-produced fruit and vegetable products does not exceed 20-25 and 50-80 %, respectively. Providing the population of the country with high-quality food is one of the priority tasks of the agro-industrial complex of Russia. The lack of fruits and vegetables for the population is covered by both domestic production and import.

Globally, the production of fruits is constantly growing. At the same time, the relevance of fruit-producing regions changed: the production of Asian fruits increased, while production in America and Europe decreased. Only one percent of global agricultural land is allocated for permanent crops and orchards. The highest percentage of agricultural land with permanent crops and orchards is in the Mediterranean region, which provided over 11% of world fruit production in 2010. In Europe and in the Central Asian region, the highest proportion of permanent crop area is in South-Eastern Europe, which is 4.4%. In the Caucasus and Turkey, this part is about 1.6%. For the subregions of Central Asia and the CIS countries, the percentage of permanent crops is even lower (with the exception of the Republic of Moldova and Tajikistan). In the regions of Europe and Central Asia, ten percent of the total agricultural land is occupied by orchards. As a result, in 2010, 54 million tons of fruit were produced, which amounted to 10% of world production. The largest regional producers, namely Turkey, Italy, Spain, and France, produce about 57% of fruit production in the region. In general, in Europe and Central Asia, the average yield in 2001-2010 was significantly higher than in the period from 1991 to 2000. South eastern Europe doubled fruit production, and production in Central Asia increased by 60% (FAO 2013).

All around the world, the technology of agricultural production involves the use of a significant amount of various pesticides, which is necessary to increase the yield and ensure production quality (Laptiev 2017).

In 2014, 55.8 thousand tons of pesticides were used in the Russian Federation. Consumption of chemical protective means amounted to 54.3 thousand tons, or 97.3% of the total amount of pesticides consumed. The consumption of insecticides was 5.3 thousand tons (decreased by 0.2 thousand tons compared to 2013), including 24.8 tons of biological insecticides or 0.47% of the total amount of insecticides consumed. The following preparations were among the most consumed ones: micro emulsion “30 Plus” (0.54 thousand tons), emulsion concentrate “Bi-58 New”, (0.36 thousand tons); microencapsulated suspension “Karate Zeon” (0.34 thousand tons), emulsion concentrate “Rogor S” (0.26 thousand tons), suspension concentrate “Borey” (0.22 thousand tons) (Govorov et al. 2015).

Moreover, vegetables and fruits are treated with pesticides during storage and transportation; in such conditions, there is always a risk of contaminating food products by pesticide residues that leads to their subsequent entry into the human
Numerous studies confirm that pesticides being biologically active substances can cause various negative effects in the case of systematic entry into the human organism (Lozowicka 2015).

It is believed that a constant entering of small quantities of pesticides into the organism from the environment with water, air, and, above all, food can be a great danger for the population. Therefore, the level of pesticide residues in food is strictly regulated in each country (ECHFS 2016; Zenkevich et al. 2002). This is one of the main indicators from the point of view of food safety. An important tool in the prevention of the negative effect on the population from the use of pesticides is monitoring of the level of their toxic residues in environmental objects, crop-growing products, feed, and food. Such monitoring enables one to accumulate data on the level of contamination of products, identify the most dangerous pesticides and their sources, as well as to develop recommendations for reducing the risk of entering pesticides into the human organism (Keikothaile et al. 2010).

For the pesticides registered on the territory of the Russian Federation, hygienic regulation of residual quantities of their active ingredients in environmental objects is based on the principle of complex hygienic regulation. According to this principle, the total amount of active ingredient of a pesticide (and its transformation products) that can enter the body from different environments should not exceed the permissible daily dose for humans (Onischenko 2010).

Pesticides belong to one of the most studied groups of chemicals used by humans for their needs (Dolzhenko et al. 2009). High level of scientific attention is explained by toxicity of pesticides to humans, domestic animals, fauna, and flora, as well as by the fact that they are intentionally introduced into the environment. The turnover of pesticides is regulated by national laws on pesticides. Along with medicinal and veterinary drugs, their introduction into practice is preceded by numerous studies and special evaluation procedures that ensure subsequent safe use. Before a new pesticide is allowed to be sold and used, it is studied for several years by the developer and then goes through state registration. After the registration, the data on the pesticide are supplemented by studies of independent scientists and state monitoring. As a result, a sufficiently large amount of data on pesticides is accumulated. These data are different in terms of availability, degree of generalization, and presentation form (Gorbatov et al. 2008).

There are many species of insects that can damage fruit and berry crops during the period of growth and fruiting. This damage decreases yield and quality of the fruits and also slows growth and development of plants. The lack of crop rotations in the case of perennial plantations contributes to the accumulation of harmful organisms. Thus, the pests should be terminated regularly starting from the moment of crop planting.

The most popular fruit trees grown in Russian orchards are apples, pears, plums, and cherries. They can be affected by a whole complex of pests, the number and harmfulness of which depend on both the varietal composition and the age of the fruit crops. The youngest trees are heavily damaged by aphids feeding on leaves and
green shoots, as well as by Diaspididae settling in the bark and branches. These pests suck sap, thereby slowing growth of plants and causing distortion of shoots.

It is almost impossible to grow a rich fruit harvest intact by pests without special measures aimed at protection of the trees. The same assures can be eliminative or preventive, mechanical, chemical, or biological. The chemical methods of pest control are considered by many to be the most effective ones. However, we should not forget that the preparations used for treating plants are not harmless and do not always completely decompose before harvesting.

In the vegetation period of 2016, the authors carried out the study of insecticide “Batraider” SC (125 g/l alpha-cypermethrin+100 g/l imidacloprid + 50 g/l clothianidin) produced by “August” company. The action of the preparation was aimed at reducing the number of different species of aphids on cherries and black currants.

Insecticide “Batraider” SC is a mixture of three active ingredients.

1. Alpha-cypermethrin (ISO), (1R cis-) S and (1S cis-)R enantiomer-isomer pair α-cyano-3-phenoxbenzyl-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate (IUPAC).

Empirical formula: C_{22}H_{19}Cl_{2}NO_{3}. Molecular weight: 416.

Alpha-cypermethrin insecticide is a representative of second-generation of synthetic pyrethroids. It consists of two cis- isomers that make up cypermethrin, which is a racemic mixture of four cis- and four trans- isomers. Pyrethroids are widely used mainly against leaf-eating insects, livestock ectoparasites, and mosquitoes. The advantages of this group of substances over other insecticides are high biological activity against insects at different stages of their development and low consumption rates (Miller and Salgado 1985).

2. Imidacloprid (ISO), 1-[(6-Chloro-3-pyridinyl) methyl]-N-nitro-4, 5-dihydro-1H-imidazol-2-amine (IUPAC).

Empirical formula: C_{9}H_{10}ClN_{5}O_{2}. Molecular weight: 255.7.

Imidacloprid is a synthetic neonicotinoid, the effectiveness of which does not depend on the ambient temperature. Within the body of insects, the substance binds to the postsynaptic receptors of the nervous system (nicotinic acetylcholine receptors), causing convulsions, paralysis, and death of pests. Imidacloprid-based substances are quite effective. The maximum effect is observed in 3-5 days after the treatment. Imidacloprid-based insecticides have both contact and systemic effects. Penetrating into the sap, these insecticides spread throughout the plant from the roots to the apices of shoots (Simon-Delso et al. 2015).

3. Clothianidin (ISO), (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine (IUPAC).

Empirical formula: C_{6}H_{8}ClN_{5}O_{2}S. Molecular weight: 249.7.

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Clothianidin is a chemical active ingredient of insecticides of contact-intestinal action, belonging to the class of neonicotinoids. It is used in agricultural industry and on personal household farms for the destruction of harmful insects. It can be used in mixture with other active ingredients (Tomlin 1997).

Alpha-cypermethrin does not penetrate into the plant, it concentrates on its surface and/or in the cuticle. Its contact and intestinal effects are very fast. Alpha-cypermethrin affects the nervous system of insects, disrupting the permeability of cell membranes and blocking sodium channels. It shows a sufficiently long residual and repellent effects.

Clothianidin is less mobile and less soluble than imidacloprid. Therefore, its connection with the surface of different parts of the plant, on which it was sprayed, is stronger. It has triple action: contact, intestinal, and systemic.

Imidacloprid, which has a solubility in water higher than that of clothianidin, is absorbed faster by the plant and travels through the tissues, making it impossible for insects to inflict significant damage on the plant. In addition, due to the gradual redistribution of this active ingredient within the crop, its constant effective concentration is maintained in the most vulnerable parts of the plant: the leaves and the spike.

These three active ingredients are effective not only on their own, but also have strong synergy, affecting various stages of nerve impulse transmission.

Alpha-cypermethrin affects sodium channels, and neonicotinoids block receptors, causing an increase in the concentration of acetylcholine in the synapse. The synergy is expressed in the simultaneous over-excitation of presynaptic and postsynaptic neurons.

The concentration of alpha-cypermethrin in preparative form of insecticide “Batrider” SC is 12.5%, the concentration of imidacloprid is 10%, clothianidin – 5%.

The aim of the present work was to study the dynamics of residual quantities of alpha-cypermethrin, imidacloprid, and clothianidin, as well as their detection in cherry (fruits and juice) and black currant (berries and juice) harvest after treating the vegetative culture with insecticide “Batrider” SC (125+100+50 g/l).

II. Materials and Methods

The work was carried out in three climatic zones: in Moscow, Tambov, and Volgograd regions of Russia.

To study the effect of the insecticide, a single spraying of vegetative plants was carried out using a knapsack sprayer with a consumption rate of working fluid of 2-3 litres/tree. The application rate for the preparation is 2.0 ml per 10 litres of water; for active substances, it is 0.25 g/ha of alpha-cypermethrin, 0.20 g/ha of imidacloprid, 0.10 g/ha of clothianidin. The authors performed 4 replicates of the experiment for each of the regions.
Sampling was carried out in accordance with the “Standardized sampling rules for agricultural products, foodstuffs, and environmental objects for the determination of residual quantities of pesticides” approved on 08/21/1979, No. 2051-79 (Zaichenko 1980). The specimens were taken separately from each plot of the considered variants. For each of the variants, one average specimen was prepared and two samples were made in the laboratory for each of the specimens.

The samples were taken separately for each of the experiment replicates, as well as for control variants untreated with pesticides. The taken samples were frozen at -18°C and stored in a freezer at the same temperature. The samples were delivered to the analytical laboratory by car equipped with a freezer (at -18°C).

The analysis of the samples for the content of alpha-cypermethrin was carried out in accordance with the “Methodical guidelines for determination of a new group of synthetic pyrethroids in plants, soil, and water of reservoirs by chromatographic methods” MG No. 4344-87 (Petrova et al. 1992).

The analysis of the samples for the content of imidacloprid was carried out in accordance with the “Methodical guidelines for determination of residual quantities of imidacloprid in water, soil, grain and straw of cereal crops, potato tops and tubers, pasture grasses, cucumbers, tomatoes, and pome fruit crops by high-performance liquid chromatography” MG 4.1.1802-03 (Tsibulskaya et al. 2007) and methodical guidelines “Determination of residual quantities of imidacloprid in apple and black currant juice, corn oil by high-performance liquid chromatography” MG 4.1.2768-10 (Dolzhenko et al. 2011).

The analysis of the samples for the content of clothianidin was carried out in accordance with the methodical guidelines “Determination of residual quantities of clothianidin in pea seeds, cabbage, tomatoes and tomato juice, fruits and juice of stone fruit crops, black currant juice and berries by high-performance liquid chromatography” MG 4.1.3511-17 (Dolzhenko et al. 2018) developed in the analytical laboratory of FSBSI All-Russian Institute of Plant Protection.

The quantitative determination of alpha-cypermethrin was performed on a “Crystal 2000 M” gas chromatograph with an electron capture detector (ECD). The length of quartz capillary column was 30 m, the size of internal diameter was 0.32 mm, the thickness of the layer of the stationary phase HP-5 was 0.25 micron. The column temperature was programmed to change from 200°C (1 min) to 260°C (4 min) at a rate of 25°C/min. The temperature of the evaporator was 260°C, the temperature of the detector was 300°C. The flow rate of the carrier gas (nitrogen) through the column G1 was 2.0 cm³/min, G4 (the rate of blowing up in the ECD) was 16 cm³/min. The division of the flow was 1:15. The dosed volume was 1 mm³.

The quantitative determination of clothianidin and imidacloprid was carried out in one sample on an ultra-performance liquid chromatograph “Acquity” (Waters, USA) with a fast-scanning UV-detector. The working wavelength was 270 nm. The column “Acquity” UPLCBEHC18 (100×2.1 mm, 1.7 μm) (Waters, USA) was used. The temperature of the column was 30±1°C. The mobile phase was acetonitrile –
0.005M H$_3$PO$_4$ taken in the ratio of 20:80. The flow rate of the eluent was 0.2 ml/min. The volume of injected sample was 10 μl.

According to Hygienic Standards, maximum residue level (MRL) of alpha-cypermethrin for stone fruits is 0.1 mg/kg, for berries – 0.07 mg/kg, MRL of imidacloprid for stone fruits is 0.5 mg/kg, for berries – 3.0 mg/kg, MRL of clothianidin for stone fruits is 0.2 mg/kg, for berries – 0.07 mg/kg (Popova 2018).

### III. Results

The costs of synthesizing new pesticides are increasing annually due to increasing strictness of sanitary-hygienic and environmental-toxicological requirements. In the United States, 21% of the total costs for pesticides were spent on the costs of work on the synthesis and screening of compounds, 6% – for toxicological assessment, 20% – for field tests, 16% – for studies of metabolism, ecotoxicology and residue analysis, and the remaining 37% – for improvement of preparative formulations, creation of industrial technology, and registration. Along with development of new pesticides, there are studies on interaction of pesticides with biological objects and their behaviour in the environment; the technologies for their effective and safe use are also developing. 39% of the total costs were spent for the study of herbicides, 34%– for insecticides, 14%– for fungicides, and 8% – for growth regulators (Zinchenko 2012). Residual amounts of pesticides in agricultural products (and products of their processing) are strictly controlled by manufacturers. The heads of enterprises are responsible for organization of control and compliance of products with hygiene requirements. Products with exceeding MRL concentrations of the substances are not allowed to be sold to the public. The use of food raw materials with a high content of pesticides for production of food is prohibited in cases where the content of toxicants in the final product cannot be reduced to permitted concentrations by industrial, culinary, or technological processing.

In order for the analytical methodology to meet its purpose, that is, to guarantee reliable and accurate analysis results, a procedure for validation of analytical methods is used, which provides the necessary and reliable information about the object of analysis and the suitability of the method for practical use (Chelovechkova et al. 2018). When carrying out validation, it is possible to identify shortcomings in a good time and to significantly improve the methodology at early stages. Accurately and thoroughly performed work is a prerequisite for reliable result, it makes one proficient in the methodology and thus significantly reduces the probability of errors in subsequent applications of validated method.

In accordance with the requirements of GOST ISO/IEC 17025-2009, the analytical methods applied in studies conducted within the framework of environmental projects, pesticide registration tests or monitoring of pesticide residues in various environments should be validated prior to their introduction into the practice of laboratories and periodically revalidated (verified) in the process of their application (EC 2016).

The results of validation of the applied methods are presented in tables 1-3.
The results of the analysis of the content of active ingredients of insecticide “Batrider” SC in plant raw materials are presented in tables 4-6. The presented results were obtained for the samples collected in Tambov region. They are almost completely consistent with the results obtained for the samples collected in other regions of the country.

IV. Discussion

The residual quantities of alpha-cypermethrin, imidacloprid, and clothianidin were not detected in the harvest of cherries and black currants (fruits and juice). Insecticide “Batrider” SC (125+100+50 g/l) created on the basis of combination the substances is a quite effective means for elimination aphids on these cultures when it is used for treatment once per season at a rate of 2.0 ml/10 l of water. The use of this insecticide provides both reliable protection of crop plantings and obtaining high-quality and safe products that can be consumed or used for production of juices and baby food.

Table 1: The recovery rate of alpha-cypermethrin extracted from the samples of leaves, fruits, and juice of the treated cultures

| Introduction rate, mg/kg | Average recovery rate, % | Standard deviation, % | Range of recovery rate, % |
|--------------------------|--------------------------|------------------------|--------------------------|
|                          | cherry                   | black currant           | cherry                   | black currant           |
|                          |                          |                        |                          |                          |
| 0.05                     | 80.94                    | 85.35                  | 4.04                     | 3.4                     | 75–88                  | 75–81                  |
| 0.5                      | 88.49                    | 88.3                   | 3.06                     | 5.51                    | 84–91                  | 80–92                  |
| average                  | 84.74                    | 86.84                  | 3.55                     | 4.45                    | 75–91                  | 75–92                  |
|                          | fruit                    |                        |                          |                          |                        |                        |
| 0.05                     | 80.58                    | 87.18                  | 6.50                     | 6.42                    | 75–89                  | 7–92                   |
| 0.5                      | 86.78                    | 88.4                   | 5.44                     | 5.97                    | 79–90                  | 82–95                  |
| average                  | 83.68                    | 87.54                  | 5.97                     | 6.19                    | 76–88                  | 76–95                  |
|                          | juice                    |                        |                          |                          |                        |                        |
| 0.05                     | 85.35                    | 82.85                  | 6.38                     | 5.70                    | 81–89                  | 79–86                  |

According to the obtained data, the average recovery rate of alpha-cypermethrin exceeds 83% for cherries and 86% for black currants both for fruits and juice, which indicates a high efficiency of the analysis method.
Table 2: The recovery rate of imidacloprid extracted from the samples of leaves, fruits, and juice of the treated cultures

| Introduction rate, mg/kg | Average recovery rate, % | Standard deviation, % | Range of recovery rate, % |
|-------------------------|--------------------------|-----------------------|---------------------------|
|                         | cherry                   | black currant         | cherry                    | black currant |
| 0.01                    | 77.2                     | 79.8                 | 1.92                      | 2.59          | 75–80 | 76–83 |
| 0.1                     | 93.0                     | 94.2                 | 3.87                      | 2.39          | 88–97 | 91–97 |
| average                 | 85.1                     | 86.8                 | 2.90                      | 2.49          | 75–97 | 76–97 |

fruits

| 0.01                    | 77.2                     | 79.0                 | 1.92                      | 3.39          | 75–80 | 75–83 |
| 0.1                     | 84.0                     | 88.0                 | 5.87                      | 3.67          | 78–93 | 84–94 |
| average                 | 80.6                     | 83.5                 | 3.90                      | 3.53          | 75–93 | 75–94 |

juice

| 0.01                    | 79.5                     | 83.5                 | 1.50                      | 1.50          | 78–81 | 82–85 |
| 0.2                     | 87.4                     | 80.5                 | 1.85                      | 1.77          | 85–90 | 79–83 |
| average                 | 86.7                     | 79.5                 | 1.455                     | 2.32          | 85–90 | 75–83 |

leaves

Imidacloprid also shows rather high recovery rate, which averages from 80 to 87%. The determination error did not exceed 3.9%.

Table 3: The recovery rate of clothianidin extracted from the samples of fruits, and juice of the treated cultures

| Introduction rate, mg/kg | Average recovery rate, % | Standard deviation, % | Range of recovery rate, % |
|-------------------------|--------------------------|-----------------------|---------------------------|
|                         | cherry                   | black currant         | cherry                    | black currant |
| 0.02                    | 77.3                     | 85.1                 | 1.68                      | 3.05          | 75–80 | 81–89 |
| 0.2                     | 82.6                     | 88.9                 | 1.85                      | 3.11          | 81–85 | 85–93 |
| average                 | 79.85                    | 87.0                 | 1.765                     | 3.08          | 75–85 | 81–93 |

fruits

| 0.02                    | 86.0                     | 78.5                 | 1.06                      | 2.87          | 85–87 | 75–83 |
| 0.2                     | 87.4                     | 80.5                 | 1.85                      | 1.77          | 85–90 | 79–83 |
| average                 | 86.7                     | 79.5                 | 1.455                     | 2.32          | 85–90 | 75–83 |

juice

The recently developed method for determination of clothianidin (Dolzhenko et al. 2018) used the modern Qu ECh ERS approach, which is widely applied for various pesticides (Komarova, 2017). Application of this method made it possible to achieve the required recovery rate of clotianidin: 79.9% and 86.7% for cherry fruits and juice and 87.0% and 79.5% for black currant fruits and juice.
Table 4: The residual amount of alpha-cypermethrin after the use of insecticide “Batrider” SC

| Sampling time, days after treatment | Analysed object | The content of the analyte in the analysed object, mg/kg |
|------------------------------------|-----------------|------------------------------------------------------|
|                                    |                 | cherry | black currant |
| treatment day                      | leaves          | 0,517  | 0,225        |
| 10                                 | fruits          | 0,255  | 0,045        |
| 20                                 | fruits          | 0,051  | 0,028        |
| 30                                 | fruits          | not detected | not detected |
| 40                                 | fruits          | not detected | not detected |
| harvest                            | fruits          | not detected | not detected |
| harvest                            | juice           | not detected | not detected |

In the process of studying the dynamics of decomposition of alpha-cypermethrin, it was found that its content was rapidly decreasing. Its value reached ½ MRL on the 20th day after the treatment. The later tests did not detect it in any of the studied cultures in all of the considered climatic zones.

Table 5: The residual amount of imidacloprid after the use of insecticide “Batrider” SC

| Sampling time, days after treatment | Analysed object | The content of the analyte in the analysed object, mg/kg |
|------------------------------------|-----------------|------------------------------------------------------|
|                                    |                 | cherry | black currant |
| treatment day                      | leaves          | 1,633  | 4,55        |
| 10                                 | fruits          | not detected | not detected |
| 20                                 | fruits          | not detected | not detected |
| 30                                 | fruits          | not detected | not detected |
| 40                                 | fruits          | not detected | not detected |
| harvest                            | fruits          | not detected | not detected |
| harvest                            | juice           | not detected | not detected |

Table 6: The residual amount of clothianidin after the use of insecticide “Batrider” SC

| Sampling time, days after treatment | Analysed object | The content of the analyte in the analysed object, mg/kg |
|------------------------------------|-----------------|------------------------------------------------------|
|                                    |                 | cherry | black currant |
| treatment day                      | leaves          | 0,812  | 2,675        |
| 10                                 | fruits          | not detected | not detected |
| 20                                 | fruits          | not detected | not detected |
Both imidacloprid and clothianidin were rapidly metabolized; therefore, these substances were detected in plant materials only on the day of treatment.

Thus, the residual quantities of the three-component insecticide “Batrider” SC are present in plant raw materials no longer than 20 days after application.

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