

RESEARCH ON TESTING NEW REMEDIES WITH SYSTEMIC FUNGICIDAL ACTION IN THE CHEMICAL MANAGEMENT OF WINTER WHEAT, CENTRAL AREA, REPUBLIC OF MOLDOVA

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Abstract

Wheat plants are subject to the impact of a complex of pathogenic agents, which include over ten species of diverse etiological and pathological nature, as key objects of economic importance, that annually cause serious damage, such as: Ustilago tritici; Tilletia caries; T. foetida; Puccinia recondita; P. glumarum; P. graminis; Erysiphe graminis; Fusarium graminearum; Septoria tritici, S. graminum; Helminthosporium tritici-repentis. The fungicidal remedies Camporo 25 EC, Custodia 320 SC and Sizaro ES fungicides have been tested against the diseases detected in winter wheat plantations. The efficiency of these new remedies with anti-fungal action was proved depending on the doses applied and the severity of the disease, as compared with the standard control. Camporo 25 EC and Custodia 320 SC, Sizaro ES fungicides are recommended as efficient chemical products in the integrated protection system of winter wheat. The frequency and intensity of the attack of pathogens, in 2021-2022, ranged between 10 and 45%, depending on environmental factors, and the biological efficiency of the new remedies tested on experimental plots as fungicides was 90-93%, at the level of the standard variant, comparing the variants and doses applied.

Key words: wheat grain; fungicides; disease, integrated protection system, biological control.

INTRODUCTION

The exploration of autumn cereal agroecosystems includes various objectives with indisputable value, to ensure the high productivity and quality of caryopses, cultivated in various agricultural production systems, on areas specialized for the cultivation of field crops, which can be affected by the instability of climatic conditions of the Republic of Moldova. Annually, various damages, in terms of phytosanitary indices, and losses of biological and agricultural production caused by the influence of the favourable impact of weather conditions on the development of complexes of harmful organisms, as parasitic agents, are reported for these crops. Special attention should be paid and detailed research should be conducted on cereal crops in impact with pathogenic agents, which cause specific diseases, with various pathological etiologies and serious consequences on wheat

plants, detected practically in all phenological stages of growth and development (Moraru, 2007; Starodub & Gheorghiev, 2008; Starodub et al., 2013).

Of particular importance in the cultivation of winter wheat are the key diseases, which appear consecutively throughout the growing season of plants caused by environmental conditions, some agrotechnological neglect and the sensitivity of wheat varieties and cultivars. Overall, the consequences of the severity of attack of pathogens causing different diseases on the vegetative organs, seriously favour the premature drying of the foliage, resulting in the reduction of photosynthesis, the retardation of growth, the formation of tiny, underdeveloped caryopses, ultimately causing lower productivity of the crop. Therefore, the foliage and the generative organs of cereal plants are subject to the attack of a complex of pathogenic agents, which includes several dozen species of diverse etiological and pathological nature, noted as

key invasive objects of economic importance that cause great damage to various cereal crops including and winter wheat, such as: *Ustilago tritici*, *Tilletia caries*, *T. contraversa*, *T. indica*, *Puccinia recondita*, *P. glumarum*, *P. anomala*, *Blumeria graminis* f. sp. *tritici* and *hordei*, *Septoria tritici*, *Septoria graminum* and *Septoria nodorum*, *Helminthosporium gramineum*, *Helminthosporium teres* etc. (Oroian & Florian, 2006; Bădărău, 2009; 2010).

The complex of protection measures for winter wheat provides for the use of all the available technological means to create unfavourable conditions for the development of pathogenic agents. Chemical treatments applied during the growing season are also very important in controlling the diseases that affect leaves and ears of wheat. The correct use of approved fungicides, by applying them at the optimal time and in doses tested during a detailed research, ensures a high effectiveness in combating the mentioned diseases (Bădărău et al., 2013; Bădărău & Nicolaesc, 2013).

Based on these facts, **the purpose and objectives of the research** carried out in 2021-2022 estimate the comparative studies on some associations of pathogenic agents in the winter wheat crop that trigger serious diseases in order to develop and implement new chemical treatments in the integrated plant protection system. New phytosanitary products, with a complex range of fungicidal action, were researched and tested to establish the biological efficiency of the products, as new remedies in combating pathogens, such as: *Erysiphe graminis*, *Puccinia recondita*, *Septoria tritici*, *Dreschlera tritici-repentis*, *Fusarium graminearum* and *Cladosporium herbarum*, under the conditions of cereal agrocenoses on productive sectors of the "Răzagro-Prim" Production Association, the central area of the Republic of Moldova, Răzeni commune, Ialoveni district.

MATERIALS AND METHODS

The success of the chemical control of diseases in autumn cereals, within the framework of intensive cultivation technologies, calls for the correct determination of the dates for applying treatments by using highly effective fungicides, the widening of the assortment of approved

chemical preparations, which are harmless to the environment. Since it is necessary to minimize productivity losses, it becomes necessary to develop effective crop protection measures that would reduce the intensity of diseases and the damage caused, using a minimum number of chemical treatments and doses of remedies. Thus, the testing of the biological efficiency of some new phytosanitary products with fungicidal action on the key diseases of winter wheat were the reason behind the goal and the main objective of the research carried out by us (Gulii Pamujac, 1992; Bădărău, 2010; Bădărău et al., 2013).

The research carried out as part of the State Testing Program of Skway Xpro control-standard preparations in comparison with the new product Cezaro ES, Camporo 25 EC and Custodia 320 SC on the pathogenic fungi, *Puccinia recondita*, *Erysiphe graminis*, *Septoria tritici*, *Dreschlera tritici-repentis*, *Fusarium graminearum* and *Cladosporium herbarum* was conducted on the territory of "Răzagro-Prim" Production Association, Ialoveni district, during the growing seasons of 2019-2021-2022. Territorially, this association owns an area of over 720 ha of arable land. According to the humidity and temperature conditions, the territory of the "Răzagro-Prim" Agricultural Production Association is part of the 2nd agroclimatic district of the Republic of Moldova and is characterized by a sum of 3200-3300°C, and the hydrothermal coefficient is 0.7-0.9. The average monthly positive air temperature is maintained during 9 months (03-11). According to multiannual data, late frosts may occur until the middle of May. Average daytime temperatures higher than 10°C are recorded over about 180 days. The amount of precipitation is 340-440 mm, of which 70 mm falls in June.

The results of the surveys carried out in several years have proven that, on the territory of "Răzagro-Prim" P.A., the conditions are very favourable both for the cultivation of winter wheat and for the development of a complex of pathogenic fungi with serious impact, such as: loose smut - *Ustilago tritici*; bunt - *Tilletia caries*, *Tilletia foetida*; brown rust - *Puccinia recondita*; yellow rust - *Puccinia glumarum*; black rust - *Puccinia graminis*; powdery mildew - *Erysiphe graminis*; fusarium head blight -

Fusarium graminearum; blotch - *Septoria tritici*, *tritici-repentis* and cladosporiosis - *Septoria graminum*; yellow spot - *Drechslera Cladosporium herbarum*.

Table 1. The scheme of the experiment for testing the biological efficiency of the fungicide Sizaro EC in combating fungal diseases in winter wheat

No	Variants of the experiment	Active ingredient	Harmful organisms	Method of use
1.	Untreated (control)	Treated with water	1. <i>Erysiphe graminis</i> ;	Two treatments per growing season
2.	Standard Skway Xpro - 1.25 l/ha	tebuconazole, 100 g/l + prothioconazole, 100 g/l + bixafen, 75 g/l	2. <i>Puccinia recondite</i> ;	
3.	Sizaro - 0.8 l/ha	prothioconazole, 125 g/l + tebuconazole, 125 g/l	3. <i>Septoria tritici</i> ;	Two treatments per growing season
4.	Sizaro - 1.0 l/ha		4. <i>Pyrenophora tritici-repentis</i> ;	
5.	Standard Nativo Pro 325 SC - 0.7 l/ha	Treated with water	5. <i>Fusarium graminearum</i> ;	Two treatments per growing season
6.	Custodia 320 SC - 0.6 l/ha	prothioconazole, 175 g/l trifloxystrobin, 150 g/l	6. <i>Cladosporium herbarum</i> .	
7.	Camporo 25 EC - 0.8 l/ha	pyraclostrobin, 170 g/l + prothioconazole, 170 g/l	1. <i>Erysiphe graminis</i> ;	Two treatments per growing season
			2. <i>Puccinia recondita</i> ;	
			3. <i>Septoria tritici</i> ;	Two treatments per growing season
			4. <i>Pyrenophora tritici repentis</i> .	



Figure 1. The experimental area with separate plots for testing the new fungicides, "Vatra-Răzăsească" P.A., Ialoveni District, 2021-2022



Figure 2. Two treatments carried out consecutively in various phenological stages on experimental plots "Vatra-Răzăsească" P.A., 2021-2022



Figure 3. Phytosanitary surveys and collecting samples for laboratory analyses, 2021-2022

Phytosanitary surveys in the areas planted with winter wheat were carried out from the fall of 2019 to 2022, where we found that in the investigated experimental sector of the

"Răzagro-Prim" P.A., significant reserves of infection were detected in the soil, for the primary and secondary inoculation on plant organs, and the environmental conditions had favourable impact on them. For cereals planted in autumn, including wheat, the treatments, during the growing season, are applied according to prognoses and warnings, but more frequently they are used according to the phenological criteria and the severity of attack by pathogens and symptoms of the detected diseases.

The experiments made in the framework of the state testing of the fungicides Cezaro EC, Camporo 25 EC and Custodia 320 SC were carried out on the winter wheat cultivar Odesscaia-269, which is vulnerable practically to all the key diseases of cereal plants. The experiment was started on 09.04.2021, according to the Latin rectangle method (Figure 1). Each variant included four replications. Plot sizes for each replication were 25 x 2 m (50 m²). The experimental plots were separated by paths of 0.4 m width, to avoid overlapping of the tested solution from one variant to another. The numbers of variants and replications were indicated with paint on boards installed in front of each plot. The plants were treated by spraying the solution with a portable sprinkler at windless morning hours (Table 1, Figures 1-3). On the experimental sector, two treatments were successively applied at an interval of one month (April 26-May 25) in the stem elongation and heading

stages. The phenological observations and the phytosanitary surveys of the development of the diseases detected in the plants grown on the experimental sector, as well as the determination of the biological efficiency of the treatments, with the remedies mentioned in Table 1, in different variants and recommended doses, were carried out periodically according to the requirements indicated by: “Methodological guidelines for testing chemical and biological plant protection products against pests, diseases and weeds in the Republic of Moldova” (2002) and “Methodological guidelines for state testing of chemical and biological products for the protection and growth stimulation of agricultural and forestry plants in the Republic of Moldova” (2012). The determination of diseases was carried out by visual and microscopic analysis, according to classic and new guidelines. The surveys to determine the severity of infection with powdery mildew, brown rust, blotch and reticulated leaf spot were carried out according to unanimously accepted methods (Bădărău & Bivol, 2009; Bădărău et al., 2013). Samples were taken 3 times during the growing season, collecting by ten plants from each individual plot, gathering them in labeled bundles, which were then analyzed in the laboratory (https://date.gov.md/ro/system/files/resources/2014-06/Catalog_2021.pdf).

To determine the severity of diseases by variants and repetitions, plant samples were collected and then analysed in the laboratory. The phytopathological research revealed the causes of the diseases, the frequency (F%) and the intensity of development (I%). Disease frequency (F%) represents the relative value of the number of affected plants or organs (n) per total number of analysed plants or organs (N) and is determined according to the formula:

$$F\% = \frac{n \times 100}{N}$$

Disease intensity (I%), which indicates the percentage to what extent a plant or organ was affected. To calculate the qualitative index of the disease intensity, the following formula was used:

$$I\% = \frac{(n_1 \cdot 1) + (n_2 \cdot 2) + (n_3 \cdot 3) + (n_4 \cdot 4)}{N \times 4}$$

where: n_1, n_2, n_3, n_4 - the number of plants or organs affected at the respective grade; N - the total number of examined plants or organs; 4 - the maximum grade of the scale.

Different scales are used to render the intensity. In our experiments, the scale with 4 grades was used to denote the disease intensity, which corresponds to certain intervals that express the percentage of the affected area, namely: 0 - visible symptoms are absent; 1 - the affected area is up to 10% of the leaf blade; 2 - the affected area is from 10 to 25%; 3 - the affected surface is from 25 to 50%; 4 - more than 50% of the surface of the organ is affected.

The biological efficiency of the tested fungicides on leaf diseases of winter wheat was calculated according to the formula:

$$B.i.\% = \frac{I.d. - I.e.}{I.d.} \times 100$$

where: **B.i.** - biological efficiency; **I.d.** - the intensity of disease development in the control; **I.e.** - the intensity of disease development in the experimental variants;

RESULTS AND DISCUSSIONS

The reduction of the damage caused by diseases in winter wheat plantations is achieved by applying a complex system of measures, which involves the use of all possibilities to prevent the attack of pathogens, starting with crop rotation, the use of healthy seeds, the correct application of soil cultivation techniques, sowing at the recommended timing and depth, the rational use of fertilizers, weed control and phytosanitary treatments with approved phytosanitary products, aimed at reducing the degree of attack by numerous pathogens, such as: *Blumeria graminis* f. sp. *tritici*, *Puccinia recondita*, *Pyrenophora tritici-repentis*, *Septoria tritici*, *Septoria graminum*, *Septoria nodorum*, *Cladosporium herbarum* *Fusarium graminearum* etc.

The agroclimatic conditions in the Central area of the Republic of Moldova during the period of research and active growth, 2020-2022, were extremely favourable for the primary infection and the active dynamic evolution of the manifestation and expansion of powdery

mildew, blotch, rusts and helminthosporiosis on the leaves and spikelets of wheat autumn and other associated diseases, which seriously affected the plants throughout the growing season, under the favourable influence of environmental factors.

The results of the preliminary and current phytosanitary surveys carried out on the winter wheat sectors indicated that, in the investigated experimental sector of the "Răzagro-Prim" P.A., significant reserves of primary and secondary inocula caused by fungal infections were detected, which caused the development of diseases throughout the growing season of the wheat crop, and the respective diseases with certain values of frequency and intensity of the attack of phytopathogenic agents are reported in Table 2, Figure 4. From the spectrum of diseases detected according to the frequency and intensity of the attack on wheat plants, especially on the vegetative organs, powdery mildew - *Erysiphe graminis* was the most common, being followed by blotch - *Septoria tritici*, *Septoria graminum*; rust - *Puccinia recondita*, *Puccinia anomala*; yellow-spot - *Pyrenophora tritici-repentis*; fusarium head blight - *Fusarium graminearum*; cladosporiosis - *Cladosporium herbarum*, mentioned in consecutive descending order according to the values of the impact indices reported during the testing period. In this context, testing the biological efficiency of the preparations Cezaro EC, Camporo 25 EC and Custodia 320 SC, with complex antifungal action against the main diseases affecting winter wheat, was the main goal and objective of the research carried out by us, together with the preventive determination of the phytosanitary status and of the economic damage threshold. The environmental conditions also contributed to a faster growth of winter wheat as well as the outbreak of the estimated specific diseases. According to the research-testing program, comparing variants and doses, individually for each disease, treatments were applied by spraying, at the same time intervals.

The comparative results of the experimental values obtained regarding the testing of the biological efficiency of the products Sizaro EC Camporo 25 EC and Custodia 320 SC, as fungicides against the pathogenic fungi *Erysiphe graminis*, *Puccinia recondita*,

Septoria tritici, *Dreschlera tritici-repentis*, *Fusarium graminearum* and *Cladosporium herbarum* are estimated in Table 2 and Figure 4.

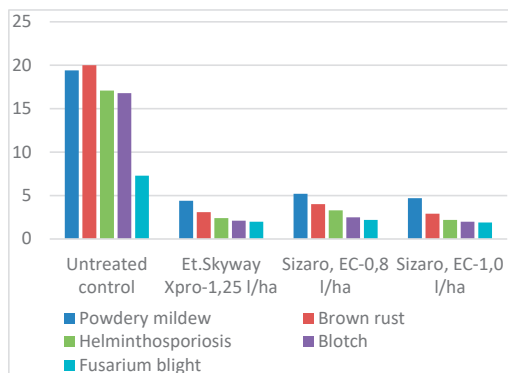


Figure 4. Comparative attack frequency by variants: untreated control, standard variant and the new tested fungicide Sizaro EC (as example)

Table 2. The results of the survey on the severity of diseases in winter wheat, central area of R. Moldova 2021-2022

Disease	Pathogen	F %	I %
Powdery mildew	<i>Erysiphe graminis</i>	44.1	28.5
Blotch	<i>Septoria tritici</i> ,	38.4	20.7
Brown rust	<i>Puccinia recondita</i>	34.5	14.0
Yellow spot	<i>Dreschlera tritici-repentis</i> ,	26.4	14.7
Fusarium blight	<i>Fusarium graminearum</i>	22.4	12.2
Cladosporiosis – black spot	<i>Cladosporium herbarum</i>	20.1	10.7

Note: F% - frequency, I% - intensity

The indices of the untreated control, where the frequency of the pathogenic attack and the intensity of the development of the diseases are indicated individually, present the following results: the frequency of the attack varied from 7.3% - fusarium blight to 22% - brown rust, and the intensity of the development of the diseases from 4.4% - fusarium blight up to 20.3% - brown rust, thus, all of them advanced within the limits from 4% to 20%. Based on the indices of the untreated control, in the treated variants, the above-mentioned fungicides were applied by spraying, as well as the standard fungicide Skway Xpro - to evaluate comparatively the biological efficiency in

winter wheat. Practically all the tested remedies reached the same values as the standard variant 92.5% (Table 3, Figure 5).

As a result of the treatment of the experimental plots with the fungicides Cezaro EC Camporo 25 EC and Custodia 320 SC, the intensity of development of powdery mildew - *Erysiphe graminis* decreased from 10.7% in the untreated

control to 1.0% after applying the first dose and 0.8% -after the second, and 0.7% after applying the standard fungicide (Skway Xpro EC 275 - 1.25 l/ha), and the biological efficiency of fungicides in combating the fungus *Blumeria graminis* f. sp. *tritici* in winter wheat was 90.6-92.5%, in comparison with the standard - 93.5%.

Table 3. Establishing the comparative biological effectiveness of the fungicides Sizaro EC, Camporo 25 EC and Custodia 320 SC, comparatively, by doses, in combating pathogenic fungi on the experimental sector planted with winter wheat, "Vatra-Răzăsească" P.A., 2021-2022

N o.	Variants of the experiment	Frequency of attack, %	Intensity of development of diseases, %	Biological efficiency, %
<i>Powdery mildew - Erysiphe graminis</i>				
1	Untreated control	19.4	10.7	0.0
2	Et. Skway Xpro – 1.25 l/ha	4.4	0.7	93.5
3	Sizaro EC – 0.8 l/ha	5.2	1.0	90.6
4	Sizaro EC – 1.0 l/ha	4.7	0.8	92.5
5	Camporo 25 EC – 1.0 l/ha	4.6	1.0	91.8
6	Custodia 320 SC -1.0 l/ha	4.7	0.9	92.6
<i>Brown rust - Puccinia recondita</i>				
1	Untreated control	20.0	14.3	0.0
2	Et. Skway Xpro – 1.25 l/ha	3.1	1.3	90.9
3	Sizaro EC – 0.8 l/ha	4.0	1.6	88.8
4	Sizaro EC – 1.0 l/ha	2.9	1.1	92.3
5	Camporo 25 EC – 1.0 l/ha	3.2	1.2	90.6
6	Custodia 320 SC – 1.0 l/ha	2.9	1.0	89.7
<i>Helminthosporiosis - Pyrenophora tritici-repentis</i>				
1	Untreated control	17.1	11.5	0.0
2	Et. Skway Xpro – 1.25 l/ha	2.4	1.0	91.3
3	Sizaro EC – 0.8 l/ha	3.3	1.3	88.7
4	Sizaro EC – 1.0 l/ha	2.2	0.9	92.2
5	Camporo 25 EC - \ 1.0 l/ha -	2.5	1.2	91.6
6	Custodia 320 SC – 1.0 l/ha	2.3	1.1	90.8
<i>Blotch - Septoria spp.</i>				
1	Untreated control	16.8	9.1	0.0
2	Et. Skway Xpro – 1.25 l/ha	2.1	0.8	91.2
3	Sizaro EC – 0.8 l/ha	2.5	1.0	89.0
4	Sizaro EC – 1.0 l/ha	2.0	0.7	92.3
5	Camporo 25 EC – 1.0 l/ha	2.2	1.1	90.8
6	Custodia 320 SC – 1.0 l/ha	2.4	0.9	91.4
<i>Fusarium blight - Fusarium graminearum</i>				
1	Untreated control	7.3	4.4	0.0
2	Et. Skway Xpro – 1.25 l/ha	2.0	0.6	86.4
3	Sizaro EC – 0.8 l/ha	2.2	0.7	84.1
4	Sizaro EC – 1.0 l/ha	1.9	0.5	88.6
5	Camporo 25 EC – 1.0 l/ha	2.3	0.8	87.3
6	Custodia 320 SC – 1.0 l/ha	2.2	0.7	86.6

As for the fungus *Puccinia recondita* the highest values were recorded in the untreated

control, with the frequency of the attack of 20.0%, and the intensity of development of the

disease of 14.3%. Among the treated variants, the frequency of the attack of brown rust was 3.1% in the standard variant (Skway Xpro- 1.25 l/ha), 4.0% after applying the first dose and 2.9% - after the second. The biological efficiency of the use of new preparations as fungicides in combating the fungus *Puccinia recondite* in winter wheat was 88.8% and 92.3%, respectively, and 90.9% if applying the standard fungicide.

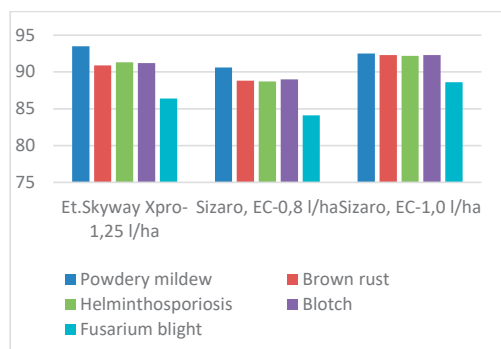


Figure 5. The comparative biological efficiency of the standard and the variant treated with the fungicide Sizaro EC for all the detected diseases

In the treated variants, the frequency of attack of helminthosporiosis was 2.4% in the standard variant - 2.2% and 3.3% in the variants treated with the new tested fungicides, and the biological efficiency of the fungicidal preparations against the fungus *Dreischlera tritici-repentis* was high, representing 91.3% in the standard variant, 88.7% and 92.2% in the tested variants. As a result of the treatments with the new remedies, the intensity of development of blotch in winter wheat decreased from 9.1% to 0.7%, as compared with 0.8% in the standard variant, and the biological efficiency of the treatments with these preparations applied to combat blotch was high, constituting 89.0-92.3% in comparison with the standard variant - 91.2%. The values obtained regarding the biological efficiency of the treatments with the tested preparations against *Fusarium graminearum* and *Cladosporium herbarum* indicated that in 2022, in the experimental sector, they developed more poorly. In the absence of the typical symptom of reddening and blackening of the wheat ears, the basic survey was carried out at the end of June (29.06.22) on the

background of the semi-hidden development of the disease, where the frequency of attack was only 7.3%, at the intensity of disease development was 4.4%. In the experimental variants, the frequency of fusarium blight was 2.2% in the variants treated with the new preparations, as compared with 2.0% in the standard variant. The intensity of the development of fusarium blight and the blackening of the wheat ears was reduced by the applied treatments, constituting 0.6% in the standard variant and 0.5-0.7% in the variants treated with the new preparations, and the biological efficiency of the treatments against the fungus *Fusarium graminearum* was 84.1% and 88.6%, respectively, as compared with 86.4% in the standard variant (Skway Xpro EC 275 - 1.25 l/ha).

The statistical processing of the obtained results shows that the biological efficiency of the treatments with the tested preparations in combating all the diseases detected in the researched winter wheat plantation, was at the level of the standard variant in both doses tested and with high values of biological efficiency, individually per disease and dose applied.

CONCLUSIONS

1. The weather conditions in the autumn of 2020 and spring-summer in the growing season of the years 2021-2022, with the exception of May and the first days of June, were unfavourable for the growth and development of winter wheat, but favourable for the primary infection and the later evolution of powdery mildew - *Erysiphe graminis*, brown rust - *Puccinia recondita*, blotch - *Septoria tritici*, helminthosporiosis - *Dreischlera tritici-repentis*, fusarium blight - *Fusarium graminearum*, cladosporiosis - *Cladosporium herbarum* and other infectious diseases detected in winter wheat, cultivated in the central area of the Republic of Moldova.
2. The comparative results of the research conducted to establish the biological efficiency of the treatments with the new remedies such as: Cezaro EC, Camporo 25 EC and Custodia 320 SC as new fungicides used for winter wheat, with a wide spectrum anti-fungal action, indicate a high efficiency of 90-93%, analysed

comparatively by doses and variants, which were at the level of the control variant - applying a standard fungicide.

3. Based on the obtained experimental results, it was proposed to include the tested preparations: Sizaro EC, Camporo 25 EC and Custodia 320 SC in the integrated protection system for winter wheat and in the State Register of phytosanitary products and fertilizers, to be applied in doses of 0.8-1.0 l/ha, two treatments per growing season, in the critical phases of contamination, according to the economic damage threshold.

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