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TESTING OF A BIOPREPARATION BASED ON *BACILLUS AMYLOLIQUEFACIENS* FOR WINTER WHEAT PROTECTION AGAINST DISEASES

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Abstract. *The results of the testing of the biological fungicide based on the bacteria Bacillus amyloliquefaciens, strain FZB24 to protect the winter wheat variety Moldova-614 from the complex of pathogens in the conditions of the Republic of Moldova. The research was carried out on the experimental field of the IGFPP of SUM. Two preparation consumption norms were tested: 2 kg/ha and 2,5 kg/ha. Used once by the spraying method. As a result, the biological efficiency against Blumeria graminis was 89,0% and 94,7%, Septoria tritici – 85,0% and 91,7%, respectively, Puccinia recondita – 94,7% and 94.9%. Reduced development of other wheat diseases may be possible.*

Keywords: Bacillus amyloliquefaciens, biological efficacy, pathogen complex, testing, winter wheat.

INTRODUCTION

Wheat (*Tríticum* L.) is the leading cereal crop in many countries, feeding more than half of the world's population. It is cultivated all over the world and ranks second after corn in terms of cultivated area. The world leaders in wheat grain production are the European Union, China and India. Also in the top five leaders are Russia and the United States. These countries account for 65% of global grain production. The world leaders in wheat yield per unit area are Germany - 7.53 t/ha, France - 6.8 t/ha, Egypt - 6.4 t/ha, China - 5.7 t/ha [1].

In Republic of Moldova, wheat sown areas in the last two decades make from 311,4 to 377,0 ha, or 20,3 - 23,6% of the total sown areas of agricultural crops. The gross grain harvest ranges from 727.7 tons in 2000 to 1 million 555 thousand tons in

2023. Wheat yield in Republic of Moldova in farms of all category's ranges from 1.9 to 4.6 t/ha [2].

Wheat is considered an unpretentious crop, preferring a moderately continental, rather warm climate. In regions that are too hot, yields are low. Gross grain yield depends on a large number of factors, including the competitiveness of the variety, weather conditions of the growing season, soil composition, agrotechnical practices, crop protection measures and others. Infection of plants with pathogenic microorganisms leads to significant yield losses. Root rots affecting plants during the whole vegetation period, powdery mildew, leaf spots, spikelet fusarium, etc. are widespread in grain crops cultivation regions. [3]. Plant protection against pests is the most important reserve for increasing production and improving its quality.

The purpose of this test is to establish the biological efficacy of biological fungicide based on the bacterium *Bacillus amyloliquefaciens,* strain FZB24 in the protection of winter wheat against a complex of diseases in the conditions of the Republic of Moldova.

MATERIALS AND METHODS

Studies were conducted in 2024 at the experimental field of the Institute of Genetics, Physiology and Plant Protection of the MSU. The object of research was a biological fungicide based on the bacterium *Bacillus amyloliquefaciens,* strain FZB24 with a titer of 1×10^{13} CFU/ml. It was used to protect winter wheat (*Tríticum aestivum* L.), variety Moldova-614 from a complex of phytopathogens: 1 - wheat mildew (*Blumeria graminis*, syn. *Erysiphe graminis*), 2 - Septoria leaf blotch (*Septoria tritici*), 3 - Brown rust (*Puccinia recondita*), 4 – Helminthosporium (*Helminthosporium* spp.), 5 - pink snow mold (*Microdochium nivale*), 6 - blight of cereals (*Fusarium graminearum*). Application is a single spraying method during the growing season.

Sowing of winter wheat was carried out in the second decade of October 2023. Laying of variants was carried out in April 2024, each of the variants in three repetitions. The area of one repetition was $25m^2$ (1m×25m), randomization method was used in placing the variants of the experiment. The testing scheme included 4 variants: 1) control - no treatment; 2) reference - Bio Force, 3 l/ha; 3) test preparation, rate of consumption 2 kg/ha; 4) test preparation, rate of consumption 2.5 kg/ha.

Prior to testing, samples were taken from different areas of the experimental field to determine the extent to which the wheat was infested by the indicated pathogens. The plants were in development phase 31-37 (BBCH) and had reached the trumpeting stage. Ten samples, each consisting of ten plants, were taken. One hundred stems were examined for snow mold, root rot, and powdery mildew. Three hundred leaves were examined to determine the presence of spot blight and brown

rust.

To determine the biological efficiency of the tested preparation in restraining the above-mentioned diseases, 3 samples of 10 plants from each repetition of the variant were taken. Plant lesion counts for spot blight, brown rust, and ear fusarium were carried out using percentage scales [4].

The obtained results were processed mathematically using one-factor analysis of variance and computer data processing methods on the ABC Pascal platform.

RESULTS AND DISCUSSIONS

Weather conditions in early autumn 2023 were not favorable for sowing and autumn vegetation of winter wheat due to insufficient moisture supply and high temperatures. In September and October, precipitation amounts amounted to 30% and 20% of the norm, respectively. The average monthly air temperature deviated from the norm by +4.0°C and +4.3°C, respectively, with anomalously high maximum temperatures of 21-31°C in the third decade of October. However, the total precipitation from January to May 2024 was 265.9 mm, significantly exceeding the norm of 194 mm (137% of the norm), which increased soil moisture reserves. This ensured the normal growth and development of wheat plants during the trumpeting phase and the beginning of the earing phase. Precipitation in June, at 65 mm (162% of the norm), contributed to the normal ripening of winter wheat and the completion of its growth cycle [5].

According to the results of surveys conducted before wheat treatment with fugicides it was found that the degree of spread of powdery mildew *Blumeria graminis* was 21.6%, the intensity of development - 4.1%. The spread of Septoria tritici septoriosis was 28.0% and the disease development was 2.3%. The spread of helminthosporiosis spot disease *Helminthosporium* sativum was weak - 5.3%, development - 0.06%. Snow mold, brown rust and helminthosporiosis root rot were not observed (Table 1).

Table 1. Infestation of wheat by pathogens before fungicide treatment

The causative agent	Disease progress, %	Disease intensity, %
Blumeria graminis	21,6	4,1
Septoria tritici	28,0	2,3
Puccinia recondita	0	0
Helminthosporium sativum	5,3	0,06
Microdochium nivale	0	0
Fusarium graminearum	0	0

Winter wheat crops were treated with the tested preparation immediately

after the first disease survey - in the BBCH 31-37 trumpeting stage of plant development. The surveys were conducted three weeks after the treatment in the BBCH 50-59 earing stage of plant development.

The surveys revealed that, over the course of three weeks after the spring disease survey, the indices of powdery mildew infestation in the control group increased significantly. The degree of spread of *B. graminis* increased from 21.4 to 21.5 tons, the percentage increased from 21.6% to 53.8%, and the intensity of development increased from 4.1% to 24.6%. Treating wheat with the tested fungicide decreased these indicators (Table 2).

Table 2. Biological efficacy of fungicide based on the bacterium *Bacillus amyloliquefaciens* in protecting winter wheat from wheat mildew *Blumeria graminis*

№	Variant	Dosa, litres, kg/ha	Disease progress, %	Disease intensity, %	Biological efficiency, %
1	Control	-	53,8	24,6	-
2	Etalon *"Bio Force"*	3,0	42,1	0,7	97,1
3	Test preparation	2,0	42,6	2,7	89,0
4	Test preparation	2,5	42,1	1,3	94,7
$HCP_{0,05}$			17,2	11,4	5,0

Therefore, our results attest that the effective treatment solution when using the rate of 2.0 kg/ha, decreased the degree of powdery mildew spread decreased to 42.6%, and the intensity of disease development to 2.7%. The biological efficiency amounted to 89.0%. Using a rate of 2.5 kg/ha resulted in a 42.1% spread and 1.3% development of the disease. The biological efficiency was 94.7%. The biological efficiency of the "Bio Force" reference material was 97.1% (Table 2).

To determine the effect of the biopreparation based on the bacterium *Bacillus amyloliquefaciens* on reducing leaf septoria, the number of infected leaves was counted in samples of ten plants. Over the course of three weeks of winter wheat vegetation after the initial disease count in the control group, the degree of septoriosis increased from 28.0% to 45.0%, and the development intensity increased from 2.3% to 13.3%. When wheat was treated with a biofungicide at a rate of 2.0 kg/ha, the spread rate of the disease was 33.2%, and the development intensity was 2.0%, with a biological efficiency of 85.0%. At a consumption rate of 2.5 kg/ha, the degree of septoriosis spread was 30.7%, the development intensity was 1.1%, and the biofungicide's biological efficiency was 91.7%. The biological efficiency of the "Bio Force" reference was 92.9 % (Table 3).

Table 3. Biological efficacy of biofungicide based on *B. amyloliquefaciens* in protecting winter wheat against Septoria leaf blotch (*Septoria tritici*)

№	Variant	Dosa, litres, kg/ha	Disease progress, %	Disease intensity, %	Biological efficiency, %
1	Control	-	45,0	13,3	-
2	Etalon *"Bio Force"*	3,0	41,6	0,94	92,9
3	Test preparation	2,0	33,2	2,0	85,0
4	Test preparation	2,5	30,7	1,1	91,7
$HCP_{0,05}$			4,9	3,1	6,1

As a result, it can be observed that no wheat brown rust (*Puccinia recondita*) infestation was detected during the first survey. Over the next three weeks of winter wheat growth, the disease spread by 33.5% in the control group and developed to an intensity of 8.1%. When wheat was treated with the tested biofungicide at a rate of 2.0 kg/ha, the spread rate of the disease was 22.6% and the development intensity was 0.43%, with a biological efficiency of 94.7%. At a consumption rate of 2.5 kg/ha, the spread rate of brown rust was 19.9%, and the development intensity was 0.41%, with a biological efficiency of 94.9% for the biofungicide. The biological efficiency of the "Bio Force" reference material was 96.3% (Table 4).

Table 4. Biological efficacy of biofungicide based on *B. amyloliquefaciens* in protecting winter wheat from brown rust (*Puccinia recondita*)

№	Variant	Dosa, litres, kg/ha	Disease progress, %	Disease intensity, %	Biological efficiency, %
1	Control	-	33,5	8,1	-
2	Etalon *"Bio Force"*	3,0	23,5	0,3	96,3
3	Test preparation	2,0	22,6	0,43	94,7
4	Test preparation	2,5	19,9	0,41	94,9
$HCP_{0,05}$			19,3	2,02	2,8

In our studies, helminthosporiosis of wheat was observed on leaves in the form of a dark brown spot caused by the pathogen Helminthosporium sativum. In the initial survey, before treatment, plant infestation by this pathogen was minimal: the degree of disease spread was 5.3%, and the intensity of development was 0.06%. After three weeks, the infestation indices remained low in the control: the degree of disease spread was 8.4%, and the intensity of development was 0.15%, both of which are below the economic threshold of harmfulness. In the treatment variants with the tested biofungicide and the "Bio Force" reference, the disease was not observed.

No snow mold (*Microdochium nivale*) or spikelet Fusarium (*F. graminearum*) pathogens were detected throughout the entire vegetation period.

CONCLUSIONS

Testing of biological fungicide based on the bacterium *Bacillus amyloliquefaciens* strain FZB24 against a complex of diseases of winter wheat under the conditions of the Republic of Moldova revealed:

1) The biopreparation significantly reduces the development of powdery mildew (*Blumeria graminis*). At a rate of 2.0 kg/ha, the biological efficiency was 89.0%; at a rate of 2.5 kg/ha, it was 94.7%.

2) The biological efficiency of the biofungicide in reducing wheat septoria (*Septoria tritici*) was 85.0% at a rate of 2.0 kg/ha and 91.7% at a rate of 2.5 kg/ha.

3) The biological efficiency of the tested biofungicide in protecting winter wheat from brown rust (*Puccinia recondita*) was 94.7% at a rate of 2.0 kg/ha and 94.9% at a rate of 2.5 kg/ha. It is possible to reduce the development of other wheat diseases.

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REFERENCES

1. Top-15 stran – proizvoditeley pshenitsy. Available: https://eldala.kz/rating/12081-top-15-stran-proizvoditeley-pshenicy.
2. Statistical Yearbook of the Republic of Moldova. *Plant production*. 2024, pp. 345-380. https://statistica.gov.md/ru/statisticeskii-ezegodnik-respubliki-moldova-vypuski-2002-2024-gg-9877_59482.html.
3. ZOSICH, A. et.al. Rekomendatsii po zashchite zernovykh kul'tur. TOO «Pervaya Agrokhimicheskaya Kompaniya». 2021, 3-25.
4. LAZARI, I. Îndrumări metodice pentru testarea produselor chimice și biologice de protecție a plantelor de dăunători, boli și buruieni în RM. *Chișinău: Tipografia Centrală*, 2002, 286 ISBN 9975-9597-3-3.
5. Arhiva meteo. Available:https://rp5.md