The effect of the ozone and the biological preparation Biofit-3 treatment on the growth of pathogenic microorganisms of wheat during storage

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Abstract. Today, wheat is one of the most common cereal crops in the world. In order for the grain to retain its qualities well, its storage conditions must be unfavorable for both insects and microorganisms. Scientists are engaged in this problem in Europe, in the United States of America, in Russia. For a number of years, Stavropol State Agrarian University has been conducting research on the effects of the Biofit-3 biological product on pathogenic microflora formed on wheat grain. Disinfection of grain by a biological preparation was carried out in the following sequence: the biological preparation was thoroughly agitated until the precipitate was dissolved; working solutions were prepared with the optimum concentration by diluting the preparation with distilled water. The solution of the biological product was evenly distributed in the mass of grain with the help of a dosing pump. Also, the experiments were conducted on the combined effects of the biological product with ozone. As a result of the experiments, the effect of the Biofit-3 preparation in combination with ozone on the suppression of the colonies of the Rhizopus, Aspergillus, Fusarium, Penicillium and Alternaria fungi in the wheat grain was found out. The article presents the optimal modes of disinfection of wheat and grain mixtures with the preparation Biofit-3 in combination with ozone.

1. Introduction

Wheat is one of the most common cereals in the world. The composition of wheat grains contains a large number of substances that help an organism in restoring and strengthening various functions, preventing diseases [1]. This product is rich in mineral compounds, such as magnesium, phosphorus, selenium, potassium, zinc, and vitamins B, E, and many amino acids. In addition, the grain has a lot of dietary fiber – 40% of daily allowance per 100 grams. However, grain quality does not always correspond to the GOST standard.

For the storage of grain in large volumes, there is a problem of migration of moisture when condensation stimulates growth of microorganisms [2]. As a result, contamination of crops with a fungal infection occurs. In turn, a fungal infection contributes to the formation of mycotoxins that form on the surface of grain and feed.

Today, about 240 toxic molds are known. Preventing the formation of mycotoxins in stored grain is an important measure in the fight for the quality of crops [3]. In order for the grain to be well preserved, its storage conditions must be unfavorable for both insects and microorganisms. Scientists in Europe, in the United States of America, in Russia are engaged in this problem [4].
The ways of dealing with the formation of mycotoxins in grain varied. Currently, common practice is aeration or ventilation of the vaults to help prevent unwanted distribution of humidity [5]. However, preventative treatment can be considered effective only if it is able to suppress the growth of fungi completely [6]. Therefore, along with preventive measures, grain is processed by physical methods, chemical and biological preparations are used to prevent and reduce grain mold.

The chemical method is based on the use of various organic and inorganic compounds that are toxic to pathogenic organisms. Modern fungicide must suppress malicious fungi without causing detrimental effects on beneficial microbiota. The new generation of preparations is typically highly reactive and toxic to certain species or related groups of microorganisms.

Today, biological preparations are becoming very popular for grain processing. Currently, more than 50 manufacturers of biological products and bacterial fertilizers are represented on the EU market [7]. The range of microbiological products for crop production is unusually wide and includes the whole range of tools necessary for growing wheat. With the proper use of biological products and all other agricultural technology requirements, the use of pesticides and sprays can be completely eliminated and the use of mineral fertilizers can be minimized [8].

Physical factors, in particular, ozone – air processing are also very popular for processing agricultural products. Such scientists as J. Palabinskis, A. Aboltinset et al [2], G. Kerch, A. Blija, R. Galoburda et al [6] and others are working in the field of ozone technologies [9].

2. Materials and methods
Taking into account the results of the scientists’ research on the influence of electrophysical factors and biological preparations for processing grain crops for several years studies have been conducted at Stavropol State Agrarian University on the effects of ozone – air flow and the Biofit-3 biological preparation on the pathogenic microflora formed on winter wheat grain.

The wheat grain processing with ozone was carried out according to the original method developed at the Educational and Scientific Testing Laboratory of Stavropol State Agrarian University. The essence of ozone processing of grain was that ozone was pumped for a certain time into the material in the ozone processing in ozone cell concentrator 7. The processing efficiency was determined by the formula

\[ D = c \cdot t, \]

where \( D \) – the dose of treatment, g·s/m²; \( c \) – ozone concentration, g/m³; \( t \) – seed treatment time (exposure), sec [10]. The ozone concentration was measured by an optical cyclone ozone gas analyzer “Cyclone-5.41”.

| Mode and processing method | Devices, preparations | Processing parameters | Exposure after processing, day |
|----------------------------|-----------------------|-----------------------|-------------------------------|
| 1 mode, Biofit-3 preparation | Biofit-3              | concentration: 1:125  |                               |
| 2 mode, Biofit-3 preparation+ ozone | Biofit-3              | concentration: 1:125  | 7, 14, 30, 60, 90, 120, 150, 180 |
| 3 mode, Biofit-3 preparation+ ozone | Ozonizer “Groza-1”  | ozone doze 28.8 g·s/m³ |                               |
| 4 mode, Biofit-3 preparation+ ozone | Ozonizer “Groza-1”  | ozone doze 28.8 g·s/m³ |                               |
| 5 mode, Biofit-3 preparation+ ozone | Ozonizer “Groza-1”  | ozone doze 28.8 g·s/m³ |                               |
| 6 mode, Biofit-3 preparation+ ozone | Ozonizer “Groza-1”  | ozone doze 28.8 g·s/m³ |                               |

Table 1. Modes of processing winter wheat grain with ozone and the Biofit-3 biological preparation.
For the biological treatment of winter wheat grain, Biofit-3 was used which contains the microbial mass of live cultures of lactic acid bacteria and bacteria of the genus Bacillus of natural origin. Biofit-3 is recommended for processing crops when laying for storage; the technical conditions of the preparation are registered in the state register of the Russian Federation. Grain disinfection with the biological product was carried out in the following sequence: before use, the biological product is thoroughly shaken until the sediment is dissolved; working solutions are prepared with the optimal concentration by diluting the preparation with distilled water. The bio preparation solution is evenly distributed over the entire grain mass using a metering pump.

Also, winter wheat grain was processed with the biological product in combination with ozone. The processing sequence is as follows: ozone-air flow → sample exposure 1 day → Biofit-3. Modes of processing grain of winter wheat are presented in Table 1.

### 3. Results

After processing in the above modes, the grain was analyzed for the presence of Rhizopus, Alternaria fungi monthly for six months. The results of the integrated impact of ozone and the biological preparation Biofit-3 on Rhizopus, Alternaria, Penicillium, Aspergillus and Fusarium fungi parasitic to the grain of winter wheat are presented in Tables 2 – 6. In particular, Table 2 shows the effect of the biological preparation Biofit-3 in various concentrations in combination with ozone on the inhibition of Rhizopus fungi colonies [10].

#### Table 2. Effect of treatments with ozone and Biofit-3 on growth of Rhizopus pathogens of winter wheat during storage (colonies/100 grains)

| Treatment modes, x | 7 | 14 | 30 | 60 | 90 | 120 | 150 | 180 | Average value |
|-------------------|---|----|----|----|----|-----|-----|-----|--------------|
| Not processed     | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 |
| 1                 | 22.0 | 6.0 | 21.0 | 15.0 | 22.0 | 23.0 | 32.0 | 32.0 | 21.6 |
| 2                 | 25.0 | 17.0 | 22.0 | 19.0 | 25.0 | 25.0 | 28.0 | 26.0 | 23.4 |
| 3                 | 24.0 | 22.0 | 21.0 | 15.4 | 19.0 | 20.0 | 29.0 | 27.0 | 22.2 |
| 4                 | 24.0 | 11.0 | 17.0 | 17.0 | 13.0 | 14.0 | 19.0 | 19.4 | 18.5 |
| Average value     | 24.0 | 16.2 | 21.2 | 18.3 | 20.8 | 21.6 | 28.6 | 27.8 | - |

**LSD_{xy, 0.95} = 1.7 (the least significant difference between variants)**

The selected processing modes of winter wheat had a different effect compared to control. Disinfection by the second and third modes did not lead to the expected result. Compared with the control (25.0 %), the difference is not significant (23.4 % in the second mode, 22.2% in the third mode) when LSD_{xy, 0.95} = 1.7. A significant difference was noted between the control and fourth mode. The infestation of the grain was almost twice reduced; the residual effect of treatment was 120 days. After this exposition, the process began of reducing ozone and the gradual reduction of the influence of Biofit-3.

Table 3 shows the results of the impact of ozone and the biological product to suppress Alternaria fungi. All modes had an impact on the reduction of infection with these fungi. The best result was achieved in the fourth mode. The number of colonies of Alternaria fungi in binning after one week was 0.4 %; then, for two months, there was a slight growth of fungi. Consequently, it is preferable to use the grain within 60 days, as then the increase of fungus infection begins.

The results presented in Table 4 provide an opportunity to assess the effect of treatment with ozone – air flow in combination with Biofit-3 on the colony of Penicillium fungi.
The table shows that the disinfection with Biofit-3 (mode 1) allows one to store wheat for 30 days, then a small development of fungus infection begins. The combined effect of ozone and Biofit-3 disinfects wheat for a long time. After processing of grain in the mode 4, the time of the aftereffects of the preparations is 120 days. The difference between control and options is significant: in control, the average value of infestation by the Penicillium fungi colonies grew by 17.1 %, in the first mode – by 8.4 %, in the second – by 9.6 %, in the third – by 9.9 %, in the fourth mode – by 7.5 %. \( \text{LSD}_{xy, 0.95} = 1.5 \). From 60 days until 120 days of grain storage, the difference of the results of processing in the fourth mode, compared to the others, is significant. The optimal parameters were achieved in the storage of grain for 90 days, in percentage terms it amounted to 2.8 % as compared to the control of 17.0 %.

Table 5 gives an idea about the impact of treatments of wheat with the biopreparation and ozone on infection of the tested samples with Aspergillus fungi colonies. The determining factors are the second, third and fourth modes, i.e., with the combined effect of ozone and the biological product. Based on the previous experiments on the effect of ozone on grain and grain mixture, it is possible to assume that the predominant influence in this case is provided by the ozone-air treatment. Compared with the control, the difference is significant in all variants. In control, the average infection rate was 23.7 %; the results were 6.6; 4.2; 2.9; 4.2 % in the first, second, third and fourth modes, respectively; \( \text{LSD}_{xy, 0.95} = 1.2 \). Therefore, the optimal is the fourth mode. The difference between the results of disinfection in this mode are significant when compared to other modes [10].

Time of effectiveness of treatments also has an impact on the condition of the grain. In particular, after two weeks of exposure, colonies of Aspergillus fungi almost completely choked in the processing of wheat grain in the second, third and fourth modes. The grain can be stored without apparent increase in fungal infection during four months; further, aftereffect of the preparations is weakened and intensive development of Aspergillus fungi begins.

### Table 3. Dynamics of the development of fungal colonies of Alternaria in winter wheat grain after treatment with ozone and Biofit-3 (colonies/100 grains)

| Treatment modes, x | Post-treatment time, day, y | Average value |
|--------------------|-----------------------------|---------------|
| Not processed      | 7  | 14 | 30 | 60 | 90 | 120 | 150 | 180 |         |
|                    | 23.0 | 23.0 | 20.0 | 20.0 | 25.0 | 24.0 | 25.0 | 25.0 | 23.1 |
| 1                  | 6.0 | 6.0 | 11.0 | 22.0 | 24.0 | 24.4 | 22.0 | 22.0 | 17.2 |
| 2                  | 4.4 | 6.0 | 11.0 | 7.0 | 21.0 | 20.6 | 22.0 | 22.0 | 14.3 |
| 3                  | 5.0 | 8.0 | 7.4 | 7.4 | 20.0 | 20.0 | 23.0 | 23.0 | 14.2 |
| 4                  | 0.4 | 6.0 | 6.0 | 11.0 | 21.0 | 21.0 | 23.0 | 23.0 | 13.9 |
| Average value      | 7.8 | 9.8 | 11.1 | 13.5 | 22.2 | 22.0 | 23.0 | 23.0 | -     |

\( \text{LSD}_{xy, 0.95} = 1.3 \)

### Table 4. Effect of treatment with ozone and Biofit-3 on the growth of Penicillium pathogens of winter wheat during storage (colonies/100 grains)

| Treatment modes, x | Post-treatment time, day, y | Average value |
|--------------------|-----------------------------|---------------|
| Not processed      | 7  | 14 | 30 | 60 | 90 | 120 | 150 | 180 |         |
|                    | 14.0 | 14.0 | 15.0 | 15.0 | 17.0 | 20.0 | 21.0 | 21.0 | 17.1 |
| 1                  | 8.0 | 7.0 | 7.0 | 8.4 | 8.4 | 8.0 | 10.0 | 10.0 | 8.4 |
| 2                  | 9.0 | 14.0 | 14.0 | 8.4 | 6.0 | 9.0 | 8.0 | 8.0 | 9.6 |
| 3                  | 16.0 | 11.0 | 14.0 | 8.4 | 8.4 | 6.0 | 8.0 | 8.0 | 9.9 |
| 4                  | 13.0 | 9.0 | 7.5 | 7.5 | 4.4 | 2.8 | 3.0 | 7.0 | 7.5 |
| Average value      | 12.0 | 11.0 | 11.5 | 8.9 | 8.5 | 9.2 | 10.8 | 10.8 | -     |

\( \text{LSD}_{xy, 0.95} = 1.5 \)
Table 5. Effect of treatment with ozone and Biofit-3 on the growth of Aspergillus pathogens of winter wheat during storage (colonies/100 grains)

| Treatment modes, x | Post-treatment time, day, y | Average value |
|--------------------|-----------------------------|---------------|
|                    | 7 | 14 | 30 | 60 | 90 | 120 | 150 | 180 |
| Not processed      | 20.0 | 20.0 | 24.0 | 25.0 | 25.0 | 25.0 | 25.0 | 25.0 | 23.7 |
| 1                  | 5.0 | 4.0 | 4.0 | 5.0 | 5.0 | 13.0 | 13.0 | 6.6 |
| 2                  | 0.4 | 0.2 | 3.0 | 5.0 | 0.0 | 0.0 | 12.0 | 13.0 | 4.2 |
| 3                  | 0.4 | 0.3 | 2.0 | 3.0 | 0.0 | 0.0 | 9.0 | 9.0 | 2.9 |
| 4                  | 3.0 | 0.0 | 5.4 | 5.4 | 0.0 | 0.0 | 10.0 | 10.0 | 4.2 |
| Average value      | 5.7 | 4.1 | 7.6 | 8.4 | 6.0 | 6.0 | 13.8 | 14.0 | - |

LSD_{xy, 0.95} = 1.2

Data on the effect of the preparation Biofit-3 in combinations with ozone on the reduction of Fusarium fungi colonies are presented in Table 6.

Table 6. Effect of treatment with ozone and Biofit-3 on the growth of Fusarium pathogens of winter wheat during storage (colonies/100 grains)

| Treatment modes, x | Post-treatment time, day, y | Average value |
|--------------------|-----------------------------|---------------|
|                    | 7 | 14 | 30 | 60 | 90 | 120 | 150 | 180 |
| Not processed      | 28.0 | 28.0 | 20.0 | 20.0 | 23.0 | 25.0 | 27.0 | 27.0 | 24.8 |
| 1                  | 6.0 | 3.4 | 6.2 | 4.0 | 1.0 | 1.0 | 0.0 | 0.0 | 2.8 |
| 2                  | 1.8 | 2.4 | 2.4 | 16.0 | 4.0 | 4.0 | 0.0 | 0.0 | 3.8 |
| 3                  | 0.4 | 2.0 | 4.0 | 11.0 | 2.0 | 3.0 | 0.0 | 0.0 | 2.8 |
| 4                  | 0.4 | 1.0 | 0.4 | 2.0 | 3.0 | 3.0 | 0.0 | 0.0 | 1.2 |
| Average value      | 7.3 | 7.2 | 6.6 | 10.6 | 6.6 | 7.2 | 5.4 | 5.4 | - |

LSD_{xy, 0.95} = 1.3

All modes to a greater or lesser extent influenced the destruction of pathogenic fungi. There has been some surge in the development of harmful fungi during processing in the second and third modes of grain exposure after 60 days. The best is the fourth mode in which the contamination by Fusarium fungi decreased and amounted to 1.2%. in the control, population colonies of these fungi amounted to 24.8 %. The difference between the control and all the selected modes is significant.

Optimal exposure of the grain after processing in different modes was noted after 7 and 30 days. After 150 days of storage, in all variants complete inhibition of colonies of the fungi was observed. However, drawing conclusions about such a long periods of storage of grain contaminated with Fusarium fungi colonies is premature, as there might be conditions suitable for reducing the growth of colonies of these fungi (e.g., reducing ambient air temperature). However, Biofit-3 in pure form and combined effects of ozone biopesticide, of course, reduce the infection of wheat by Fusarium fungi colonies.

4. Conclusion

Thus, the conducted experiments suggest that the complex treatment of winter wheat grain with ozone and the Biofit-3 biological product allows one to reduce its contamination with harmful fungi. Recommended disinfection modes are as follows:

• colonies of Fusarium fungi: the concentration of the preparation Biofit-3 is 1: 500, the dose of ozone is 28.8 g·s/m³. Shelf life of grain is 7–180 days.

• Alternaria fungi colonies: the concentration of the Biofit-3 preparation is 1: 250, the ozone dose is 28.8 g·s/m³. Store grain for no more than 60 days.
• Rhizopus fungi colonies: the concentration of the Biofit-3 preparation is 1: 125. The time of grain exposure after treatment is 14 days.
• Penicillium fungi colonies: the concentration of the Biofit-3 preparation is 1: 500, the ozone dose is 28.8 g·s/m³. It is better to use grain for 60–120 days.
• colonies of Aspergillus fungi: the concentration of the Biofit-3 preparation is 1: 250, the ozone dose is 28.8 g·s/m³. The optimum storage time is from 14 to 120 days[10].

References
[1] Shevchenko A A, Saprunova E A and Shkhalakhov R S 2003 The influence of ozone on grain crops Physical and technical issues of creating new technologies in the agro-industrial complex: Proc. of Int. conf. of SSAU pp 645–646 (in Russian)
[2] Aboltins A, Palabinskis J and Lauva A 2010 Grain active ventilation using ozonized air Acta-Horticulturae
[3] Emelyanov S A, Mandra Y A, Gudiev O Y, Maznitsyna L V and Korostylev S A 2016 Effects of anthropogenic environmental and food safety Research J. of Pharmaceutical, Biological and Chemical Sciences 7(3) 2565–2569
[4] Avdeeva V N, Antonov S N, Molchanov A G and Devederkin I V 2017 Disinfecting of winter wheat grain with electrophysical factors Engineering for rural development pp 323–327 (Jelgava, Latvia)
[5] Trubina I A, Scorokbina E A, Zakotin V E and Bezgina J A 2016 Methodological basis of food production for special purposes Research J. of Pharmaceutical, Biological and Chemical Sciences 7(1) 1621–1625
[6] Kince T, Galoburda R, Klava D, Blija A and Kerch G 2017 Effect of processing on microbial safety, total phenolic content and radical scavenging activity of germinated hull-less barley flakes J. of Food, Agriculture and Environment
[7] Shutko A P, Shek E G, Tuturzhans L V, Mikhno L A and Ustimov D V 2018 Mycobiota grains of winter wheat, depending on the fungicidal treatment Research J. of pharmaceutical, biological and chemical sciences 9(6) 1641–1644
[8] Okrut S V, Mandra Y A, Stepanenko E E, Zelenskaya T G and Gudiev O Yu 2018 Application of physical and chemical methods in the assessment of the ecological state of the environment of urbanized areas Research J. of pharmaceutical, biological and chemical sciences 9(4) 660–665
[9] Pashkova E V, Bezgina Yu A, Shipulya A N, Volosova E V and Mikhno L A 2018 Growth-stimulating preparations based on lactose-containing raw materials into technologies of grain crops cultivation Research J. of pharmaceutical, biological and chemical sciences 9(4) 1105–1109
[10] Avdeeva V N 2009 The use of ecological methods for the suppression of pathogenetic microflora of winter wheat grain during storage, candidate dissertation (Stavropol, 2009)